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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/705, C12N 15/12, 15/58, A61K 38/17, 38/48, G01N 33/68 — A2

(11) International Publication Number: **WO 97/40072**

(43) International Publication Date: 30 October 1997 (30.10.97)

(21) International Application Number: PCT/GB97/01067

(22) International Filing Date: 16 April 1997 (16.04.97)

(30) Priority Data:
9608130.2 19 April 1996 (19.04.96) GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ADAM PROTEINS AND USES THEREOF

(57) Abstract

Described are ADAM 12 proteins, species variants, homologues, allelic forms, mutant forms, derivatives, muteins and equivalents thereof, and various individual domains of ADAM 12 proteins, various domain combinations, inhibitors thereof and various forms of therapy, diagnosis and prophylaxis based thereon. Various therapeutic, diagnostic and prophylatic applications of proteins of the ADAM (reprolysin) family in general, as well as their individual domains, domain combinations, inhibitors and other materials based thereon, are also described.

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ADAM PROTEINS AND USES THEREOF

Background of the invention

The present invention relates to ADAM 12 proteins, to species variants, homologues, allelic forms, mutant forms, derivatives, muteins and equivalents thereof, to various individual domains of ADAM 12 proteins and various domain combinations, to inhibitors thereof and to various forms of therapy, diagnosis and prophylaxis based thereon. The invention also relates to various therapeutic, diagnostic and prophylactic applications of proteins of the ADAM (reprolysin) family in general, as well as their individual domains, domain combinations, inhibitors and other materials based thereon.

The ADAM family is a term of art defining a recently recognized and widely distributed family of membrane proteins which contain both a metalloprotease domain and a disintegrin domain (see e.g. Wolfsberg et al. (1995), J. Cell Biol., Vol. 131(2), pages 275-278 and Wolfsberg et al. (1995), Developmental Biology, Vol. 169, pages 378-383). They are closely related to certain snake venom proteins with which they have been grouped as a single family, the *reprolysins* (Bjarnason and Fox (1994), Pharmac. Ther. Vol. 62, pages 325-372).

The ADAM family is also recognized as a grouping in an independent classification scheme representing a family which at least partially overlaps with the reprolysin family. The reprolysin family of metalloproteinases are a well characterised group of enzymes present in snake venom. The family has been divided into four classes based upon the characteristic domains present (Bjarnason and Fox (1994), Pharmac. Ther. Vol. 62, pages 325-372; Rawlings and Barrett (1995), Evolutionary families of metallopeptidases, In: A.J.Barrett (ed.), Proteolytic enzymes: Aspartic and metallopeptidases, pp. 183-228, London: Academic Press Inc.).

Representatives of class I contains a proteinase domain only, those of class II a proteinase and disintegrin-like domain, whereas those of class III contain proteinase, disintegrin-like and cysteine rich domains. The class IV reprolysins are similar to the class III molecules with the addition of a lectin-like domain at the C terminus (Bjarnason and Fox, Snake venom metalloendopeptidases: Reprolysins. In: A.J.Barrett (ed.), Proteolytic enzymes: Aspartic and metallopeptidases, pp. 345-368, London: Academic Press Inc.).

Members of this family of enzymes have been shown to degrade basement membranes, having a potent hydrolytic

activity against type IV collagen, laminin and fibronectin.

The ADAM proteins exhibit structural affinities with inter alia the class III and class IV reprolysins. As used herein, the terms ADAM, ADAM protein(s) and ADAM family are used in a broad sense to correspond to the reprolysins as defined by Bjarnason and Fox (1994), Pharmac. Ther. Vol. 62, pages 325-372 and Bjarnason and Fox, Snake venom metalloendopeptidases: Reprolysins. In: A.J.Barrett (ed.), Proteolytic enzymes: Aspartic and metallopeptidases, pp. 345-368, London: Academic Press Inc.). These terms therefore encompass related metalloproteinases of snake origin. In particular, the terms ADAM, ADAM protein and ADAM family refer to the ADAM family as defined by Wolfsberg et al. (1995), J. Cell Biol., Vol. 131(2), pages 275-278 and Wolfsberg et al. (1995), Developmental Biology, Vol. 169, pages 378-383 together with classes P-III and P-IV of the venom proteinases.

The terms ADAM, ADAM protein(s) and ADAM family are also intended to be interpreted herein in a narrower sense to correspond to the family defined by Wolfsberg et al. (1995), J. Cell Biol., Vol. 131(2), pages 275-278 and Wolfsberg et al. (1995), Developmental Biology, Vol. 169, pages 378-383.

One striking feature of the ADAM (reprolysin) family is mosaicism: multiple structural domains are present at both the DNA and protein level. These may include a metalloprotease-like domain, which may or may not be proteolytically active. Proteolytically active ADAM{met} domains may be characterized by the presence of a HEXXH motif (characteristic of zinc-dependent metallo-endopeptidases). ADAM{met} domains which lack this motif may have a different activity (such as a different proteolytic activity/substrate specificity), may act as an effector sink (and so act as a regulatory element), or fulfil a structural role in the context of a multidomain ADAM protein (perhaps to effect signalling across a membrane). Different splicing pathways may also generate ADAMs having different ADAM{met} domains, and so the presence of an active ADAM{met} may dependent on a particular tissue-specific splicing pathway.

The ADAM proteins may also include a disintegrin-like domain (which may or may not be a ligand for integrins or other receptors), a potential fusogenic motif or domain (which may or may not be fusogenic, and which may form part of a high-cysteine region which may be associated with the function of the disintegrin-like domain), an EGF-like repeat-containing domain (which also forms part of the cysteine rich region) and a cytoplasmic tail domain

containing a transmembrane subdomain. Not all ADAMs have a potential fusogenic motif/domain.

In many ADAM proteins, the metalloprotease-like domain (hereinafter abbreviated ADAM{met}) may contain the consensus sequence HEXGHNLGXXHD. Structural studies suggest that the three histidines (underlined) bind zinc.

The disintegrin-like domain (hereinafter abbreviated ADAM{dis}) may be a ligand for integrins or other receptors. Different ADAMs may interact with different integrins and/or other receptors. Only a subset of ADAMs may be functional adhesion molecules.

The disintegrin loops of all ADAMs and P-III SVMPs contain an extra cysteine as compared to those of P-II SVMPs. This cysteine, which provides the disintegrin-like domain with an odd number of cysteines, may be free, or it may form a disulfide bond with a cysteine in the cysteine-rich domain, which also contain an odd number of cysteines. The unpaired cysteine could be available for dimer (homo- or heterodimer) formation. Recent evidence suggests that disintegrin-like domains which contain this extra cysteine are functional adhesion molecules.

The cytoplasmic tails are unusually rich in proline,

serine, glutamic acid, and/or lysine. None of the tails show obvious sequence similarity to other proteins. Given the roles of the cytoplasmic domains of other plasma membrane proteins, the tails of the ADAMs may be involved in oligomerization or signalling.

Regulation of ADAM activities is complex. Transcription of ADAM mRNA is modulated both positionally and temporally. For example, although some ADAM mRNA are testis-specific, others are found in a variety of tissues. In addition, transcription of ADAMs 1-6 in the testis is developmentally regulated. Splicing of ADAM transcripts is also regulated. Many ADAMs are composed of multiple small exons and alternatively spliced forms of ADAM II have been proposed. The two isoforms of monkey ADAM 1 could be due either to alternative splicing or to multiple genes. ADAMs may also be regulated at the protein level. ADAMs may form combinations of dimers or oligomers. For example, as ADAMs 1 and 4 show identical patterns of tissue distribution, there may be ADAM 1/4 complexes in some cell types. Proteolytic cleavage between domains may regulate certain functions, as many SVMPs, and possibly guinea pig fertilin ß, are proteolyzed at interdomain boundaries. Many ADAMs and SVMPs contain di- or tetrabasic residues between domains which could be recognized by interdomainases (for example, subtilisin-like proteases). As in the case of

viral fusion proteins, proteolytic processing may be necessary to render fusion competence. Guinea pig fertilin α and ß are processed during sperm maturation, but EAPI (ADAM 7) and MS2 (ADAM 8) have been reported only as unprocessed proteins. Regulation of ADAM metalloprotease activity may also occur by a cysteine-switch mechanism, in which a prodomain cysteine ligands the active site zinc and maintains it in an inactive state. ADAMs which encode the metalloprotease active site residues contain a cysteine in their prodomain which is not present in ADAMs which lack the zinc-liganding histidines.

Summary of the invention

According to the present invention there is provided an isolated ADAM 12 protein.

The term ADAM 12 protein is used herein to refer to the human ADAM 12 protein (hereinafter *h*ADAM 12), as represented by the amino acid sequence shown in Figure 2, together with species variants, allelic and mutant forms thereof.

The term ADAM 12 is also used herein to refer to a particular set of members of the ADAM family having amino acid sequences exhibiting high degrees of similarity with

that of Figure 2, including allelic and mutant forms thereof.

Thus, the term ADAM 12 protein encompasses *h*ADAM 12 and all homologues or species variants thereof (for example, species variants occurring in other organisms, particularly in other mammals). In particular, the term ADAM 12 as used herein embraces the bovine homologue or species variant (hereinafter *b*ADAM 12).

However, preferred according to the invention is *h*ADAM 12 protein and its allelic and/or mutant forms and derivatives.

The term ADAM 12 may also encompass variant protein forms generated by alternative splicing pathways ("splicing variants") which arise during expression of the corresponding structural gene(s). Thus, in an extended sense the term ADAM 12 defines an ADAM 12 protein complex, this complex being that constellation of proteins derived by expression of ADAM 12 structural gene(s) via each of the various different splicing pathways in each of the various different organisms in which it occurs, including allelic and mutant forms thereof.

The term isolated is used herein to indicate that the

isolated protein or substance exists in a physical milieu distinct from that in which it occurs in nature. For example, the isolated protein or substance may be substantially isolated (for example purified) with respect to the complex cellular milieu in which it naturally occurs. When purified material of the invention is specified herein the absolute level of purity is not critical and those skilled in the art can readily determine appropriate levels of purity according to the use to which the material (e.g. ADAM protein) is to be put.

In many circumstances, the isolated material (e.g. protein) will form part of a composition (for example a more or less crude extract containing many other proteins and substances), buffer system or pharmaceutical excipient, which may for example contain other components (including other proteins, such as albumin).

In other circumstances, the isolated protein may be purified to essential homogeneity, for example as determined by PAGE or column chromotography (for example HPLC).

In preferred embodiments, the isolated ADAM protein species of the invention is essentially the sole ADAM protein in a given composition. Particularly preferred are compositions

in which the ADAM 12 protein (or homologues, muteins, derivatives or equivalents thereof) is the sole representative of the ADAM family (for example, the sole active ingredient in a pharmaceutical composition).

The designations ADAM 12{pro}, ADAM 12{met}, ADAM 12{dis}, ADAM 12{fus}, ADAM 12{egf} and ADAM 12{tmt} are abbreviations for the pro-domain, metalloprotease-like domain, disintegrin-like domain, fusogenic-like motif or domain, EGF-like domain and transmembrane-cytoplasmic tail domains of the ADAM 12 protein, respectively.

The protein of the invention preferably comprises or consists essentially of: (a) ADAM 12{pro}; and/or (b) ADAM 12{met}; and/or (c) ADAM 12{dis}; and/or (d) ADAM 12{fus}; and/or (e) ADAM 12{egf}; and/or (f) ADAM 12{tmt}.

Thus, the invention specifically contemplates all individual domains of the ADAM 12 protein as separate and independent entities, as well as all combinations thereof and all combinations of subsets thereof. In this context, a subset is a group of some (but not all) ADAM domains as hereinbefore described.

As explained above, the ADAM 12{met} domain may not be an active protease, and the ADAM 12{dis} domain may not act as

a functional disintegrin (e.g. may not interact with integrins or other receptors). The ADAM 12{fus} domain is not necessarily fusogenic.

Preferably, the isolated protein comprises or consists essentially of: (a) ADAM 12{pro}; or (b) ADAM 12{met}; or (c) ADAM 12{pro} and ADAM 12{met}; or (d) ADAM 12{pro}, ADAM 12{met} and ADAM 12{dis}; or (e) ADAM 12{met} and ADAM 12{dis}; or (f) ADAM 12{dis}; or (g) ADAM 12{egf}; or (h) ADAM 12{met} and ADAM 12{egf}; or (i) ADAM 12{met}, ADAM 12{dis} and ADAM 12{egf}; or (j) ADAM 12{pro}, ADAM 12{met}, ADAM 12{dis} and ADAM 12{egf};or (k) ADAM 12{dis} and ADAM 12{fus}; or (l) ADAM 12{met}, ADAM 12{dis} and ADAM 12{fus}. Such domain sequences may or may not occur in nature.

Some or all of these particular combinations may be functionally complementary in that the conjunction of the selected domains promotes or enables (either directly or indirectly via one or more mediators) a functional interaction therebetween.

For example and without wishing to be bound by theory, it is believed that the ADAM{pro} and ADAM{met} domains together comprise a zymogen activated by proteolysis at the interdomain boundary. The ADAM{pro} domain may thus act as

a cysteine switch, co-ordinating the active site zinc atom via a cysteine residue thus interfering with binding of a water molecule and rendering the ADAM{met} domain inactive.

By way of further example, the ADAM{met} domain may be involved in autoactivation by a proteolytic event, and a high-cysteine sequence context in which the ADAM{fus} may be located and/or the ADAM{egf} domain may function as inhibitors of ADAM{met}.

By way of further example, the cysteine switch may also be activated by cleavage at the ADAM{pro}-ADAM{met} interdomain boundary by a matrix metalloprotease (MMP), for example a membrane-bound MMP or gelatinase A. Thus, one class of ADAM{met} inhibitors would be inhibitors of the activating MMPs.

Where the isolated protein of the invention comprises two or more particular domains, it is to be understood that these domains can be present as the products of cotranslation from a corresponding DNA (e.g. a cDNA) in which the corresponding structural genes are arranged in tandem, or can be the products of fusion, crosslinking (e.g. chemical or immunological cross linking), binding or other associative processes conducted on mixtures of individual domains (which may have been independently

purified or co-purified).

Preferably, multidomain proteins according to the invention are the products of the expression of recombinant DNA molecules in which the structural genes for individual ADAM domains have been operably linked in various preselected patterns. In this context, "operably linked" means that the structural genes for each domain can give rise to a messenger RNA which can be translated to yield the fused ADAM protein domains. This is conveniently achieved by preparing a collection of DNA cassettes consisting essentially of the coding sequences for each domain, which cassettes are bounded by one or more restriction endonuclease sites (or cloning sites) to facilitate their manipulation in vitro using standard cloning techniques.

Multidomain ADAM proteins of the invention may comprise one or more heterogenous domains. In this context, a heterogenous domain is one which is derived from (or corresponds to) a different ADAM to the other domain(s) with which it is associated. Thus, the invention covers ADAM proteins in which some or all of the constituent domains are derived from different organisms (e.g. different species).

The invention also contemplates a DNA fragment or cassette

consisting essentially of DNA encoding the ADAM{pro}, ADAM{met}, ADAM{dis}, ADAM{fus}, ADAM{egf} or ADAM{tmt} domain, the fragment or cassette optionally being bounded by one or more restriction endonuclease cleavage sites or cloning sites.

Preferably, such ADAM DNA cassettes are ADAM 12 DNA cassettes (for example *h*ADAM 12 or *b*ADAM 12 cassettes).

The invention also contemplates concatenated domain cassettes, as well as mutant ADAM structural genes which have cloning sites (e.g. one or more restriction endonuclease cleavage sites) located in one or more interdomain boundary regions.

The invention also embraces homologues, derivatives, muteins or equivalents of the protein (particularly the ADAM 12 protein) of the invention. An example of an equivalent of the ADAM 12 protein are proteins identified by probing with *h*ADAM 12 or *b*ADAM 12 nucleic acid (e.g. having a sequence corresponding to all or a portion of that shown in Fig. 2) according to techniques known to those skilled in the art. As used in this context, *h*ADAM 12 or *b*ADAM 12 nucleic acid is that which corresponds to any *h*ADAM 12 or *b*ADAM 12 structural gene (including for example alleles or mutant forms of *h*ADAM 12 or *b*ADAM 12, for

example nucleic acids in which nucleotide residues have been altered in the light of the codon usage in the organism being probed).

The term homologue is used herein in two distinct senses. It is used sensu stricto to define the corresponding protein from a different organism (i.e. a *species variant*), in which case there is a direct evolutionary relationship between the protein and its homologue. This is reflected in a structural and functional equivalence, the protein and its homologue performing the same role in each organism.

The term homologue is also used herein sensu lato to define a protein which is structurally *similar* (i.e. not necessarily related and/or structurally and functionally equivalent) to a given (reference) protein. In this sense, homology is recognized on the basis of purely structural criteria by the presence of amino acid sequence identities and/or conservative amino acid changes (as set out by Dayoff et al., *Atlas of protein structure* vol. 5, National BioMed Res Fd'n, Washington D.C., 1979).

For the purposes of the invention, homologues may be recognized as those proteins the corresponding DNAs of which are capable of specifically or selectively cross-hybridizing, or which can cross-hybridize under stringent

hybridization conditions.

The term selectively or specifically (cross)hybridizable in this context indicates that the sequences of the corresponding ssDNAs are such that binding to a unique (or small class) of homologous sequences can be obtained under more or less stringent hybridization conditions. This method of the invention is not dependent on any particular hybridization conditions, which can readily be determined by the skilled worker (e.g. by routine trial and error or on the basis of thermodynamic considerations).

Preferably, the homologues, derivatives, muteins or equivalents of the ADAM 12 protein of the invention have at least 20% identity with the *h*ADAM 12 (Fig. 2) or *b*ADAM 12 amino acid sequence.

The homologues, derivatives, muteins or equivalents of the individual ADAM 12 domains (or domain combinations) of the invention have at least 20% identity with the amino acid sequences of the *respective h*ADAM 12 (Fig. 2) or *b*ADAM 12 domains.

Particularly preferred are homologues, derivatives, muteins or equivalents having at least 30% identity, for example at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 80%, 85%,

90%, 95% or 98% identity with these ADAM 12 amino acid sequences.

Particularly preferred are homologues, derivatives, muteins or equivalents of isolated ADAM domains or domain combinations having at least 30% identity, for example at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 80%, 85%, 90%, 95% or 98% identity with the corresponding ADAM 12 domain amino acid sequences.

The homologues, derivatives, muteins or equivalents of the ADAM 12 protein (or individual domain)s) thereof) of the invention may have at least 25% homology with the *h*ADAM 12 (Fig. 2) or *b*ADAM 12 amino acid sequence (or with the amino acid sequences or the corresponding domains).

Particularly preferred are homologues, derivatives, muteins or equivalents having at least 30% homology, for example at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 80%, 85%, 90%, 95% or 98% homology with these ADAM 12 (or ADAM 12 domain) amino acid sequences.

Preferred homologues of the ADAM 12 protein are the *b*ADAM 12 and *h*ADAM 12 proteins, together with their allelic and mutant forms as well as the alternative splice pathway-derived variants.

Thus, the homologues, derivatives, muteins or equivalents of the invention are to be understood as applying to the entire mosaic ADAM protein as well as to its individual domains/domain subset combinations.

The term derivative as applied herein to the proteins of the invention is used to define proteins which are modified versions of the proteins of the invention. Such derivatives may include fusion proteins, in which the proteins of the invention have been fused to one or more different proteins or peptides (for example an antibody or a protein domain conferring a biochemical activity, such as enzymic or conjugative activity, to act as a label, or to facilitate purification).

The derivatives may also be products of synthetic processes which use a protein of the invention (for example, an ADAM 12 protein, e.g. *h*ADAM 12 or *b*ADAM 12) as a starting material or reactant.

The term mutein is used herein to define proteins that are mutant forms of the proteins of the invention, i.e. proteins in which one or more amino acids have been added, deleted or substituted. The muteins of the invention therefore include truncates (e.g. ADAM 12 truncates), fusion proteins (for example, fusions of the ADAM 12

protein or one or more domains thereof with another protein, such as an immunoglobulin, a peptide toxin, a receptor or enzyme) and a variety of different combinations of ADAM domains. Particularly preferred truncates are those which lack the ADAM{tmt} domain.

Also particularly preferred are proteins (e.g. fusion proteins) which comprise "shuffled" ADAM (e.g. ADAM 12) domains. Such proteins are hereinafter referred to as shuffled ADAM proteins. Shuffled ADAM proteins comprise or consist essentially of arrays of two or more ADAM domains (e.g. two or more cotranslated ADAM domains) in a sequence other than that found in the canonical ADAM{pro}-ADAM{met}-ADAM{dis}-ADAM{fus}-ADAM{egf}-ADAM{tmt} sequence.

Shuffled ADAM proteins are conveniently produced by processes involving the use of the DNA fragments or cassettes consisting essentially of DNA encoding the ADAM{pro}, ADAM{met}, ADAM{dis}, ADAM{fus}, ADAM{egf} or ADAM{tmt} domain of the invention, which may optionally be bounded by one or more restriction endonuclease cleavage sites or cloning sites. The shuffled ADAM proteins may also be produced by processes involving the concatenated domain cassettes of the invention, or mutant ADAM structural genes which have cloning sites (e.g. one or more restriction endonuclease cleavage sites) located in one or more

interdomain boundary regions.

The muteins may also include proteins in which mutations have been introduced which effectively promote or impair one or more activities of the protein, for example mutations which promote or impair the function of a catalytic site or an effector binding site.

The muteins of the invention may also comprise ADAM proteins in which one or more interdomain boundaries have been replaced with other sequences, for example with sequences encoding proteolytic cleavage sites.

Other preferred mutant forms are truncates consisting essentially of the catalytic, binding, effector or receptor domains of the various ADAM domains. Such domains may be identified and isolated by performing mutagenesis followed by functional assay and/or by the identification of canonical or conserved sequence motifs.

The term equivalent as used herein and applied to the proteins of the invention defines proteins which exhibit substantially the same functions as those of the proteins of the invention while differing in structure (i.e. amino-acid sequence). Such equivalents may be generated for example by identifying sequences of functional importance

(e.g. by identifying conserved or canonical sequences or by mutagenesis followed by functional assay), selecting an amino acid sequence on that basis and then synthesising a peptide based on the selected amino acid sequence. Such synthesis can be achieved by any of many different methods known in the art, including solid phase peptide synthesis (to generate synthetic peptides) and the assembly (and subsequent cloning) of oligonucleotides.

The homologues, fragments, muteins, equivalents or derivatives of the proteins of the invention may also be defined as those proteins which cross-react with antibodies to the proteins of the invention, and in particular which cross-react with antibodies directed against *h*ADAM 12 or *b*ADAM 12 (or any particular domain thereof).

The ADAM proteins of the invention may be provided in the form of oligomers, tetramers, trimers, dimers or monomers. Preferably, the ADAM proteins of the invention are provided in the form of homotetramers, homotrimers or homodimers. However, the ADAM proteins may also be provided in the form of heterotetramers, heterotrimers or heterodimers. Preferably at least one member of the heteromeric forms is an ADAM 12 monomer (for example, in association with ADAM 11 and/or ADAM 10).

The invention also contemplates pharmaceutical compositions comprising any of the proteins of the invention or homologues, derivatives, muteins or equivalents thereof.

A pharmaceutical composition is a solid, liquid or gaseous composition in a form, concentration and level of purity suitable for administration to an individual upon which administration it can elicit physiological changes in a patient (e.g. to a human or animal patient).

In another aspect the invention relates to a pharmaceutical composition comprising an ADAM protein or an isolated ADAM protein which is: (a) for use in therapy, diagnosis or prophylaxis; or (b) in a pharmaceutical excipient, a unit dosage form or in a form suitable for local or systemic administration.

The generic terms ADAM and ADAM protein are also terms of art, and are used herein in several senses as described above. The terms therefore define inter alia a family of proteins sharing sequence similarity or homology, and include the proteins described in e.g. Wolfsberg et al., 1995, J. Cell Biol., Vol. 131(2), pages 275-278. Thus, as used herein, the terms ADAM, ADAM protein and ADAM family are used in an extended sense to correspond to the reprolysins as defined by Bjarnason and Fox (1994),

Pharmac. Ther. Vol. 62, pages 325-372 and Bjarnason and Fox, Snake venom metalloendopeptidases: Reprolysins. In: A.J.Barrett (ed.), Proteolytic enzymes: Aspartic and metallopeptidases, pp. 345-368, London: Academic Press Inc.). These terms therefore encompass related metalloproteinases of snake origin. In particular, the terms ADAM, ADAM protein and ADAM family refer to the ADAM family as defined by Wolfsberg et al. (1995), J. Cell Biol., Vol. 131(2), pages 275-278 and Wolfsberg et al. (1995), Developmental Biology, Vol. 169, pages 378-383 together with classes P-III and P-IV of the venom proteinases.

The terms ADAM, ADAM protein and ADAM family are also used herein in a narrower sense to correspond to the family defined by Wolfsberg et al. (1995), J. Cell Biol., Vol. 131(2), pages 275-278 and Wolfsberg et al. (1995), Developmental Biology, Vol. 169, pages 378-383.

The term family, as applied to proteins, is used herein to indicate a group of proteins which share substantial sequence similarities, either at the level of the primary sequence of the proteins themselves, or at the level of the DNA encoding them. The sequence similarities may extend over the entire protein/gene, or may be limited to particular regions or domains. Similarities may be based on

nucleotide/amino acid sequence identity as well as similarity (for example, those skilled in the art recognize certain amino acids as similar, and identify differences based on switches of similar amino acids as conservative changes). Some members of a protein family may be related in the sense that they share a common evolutionary ancestry, and such related proteins may herein be referred to as homologues. The members of a protein family do not necessarily share the same biochemical properties or biological functions, though their similarities are usually reflected in common functional features (such as effector binding sites and substrates). The criteria by which protein families are recognized are well-known in the art, and include computer analysis of large collections of sequences at the level of DNA and protein as well as biochemical techniques such as hybridization analysis and enzymatic assays (see for example Lipman and Pearson, Science, Vol. 227, pages 1435-1441).

According to a further aspect of the present invention there is provided isolated protein consisting essentially of: (a) ADAM{pro}; and/or (b) ADAM{met}; and/or (c) ADAM{dis}; and/or (d) ADAM{fus}; and/or (e) ADAM{egf}; and/or (f) ADAM{tmt}, the isolated protein for example forming part of a pharmaceutical composition or for use in therapy, diagnosis or prophylaxis.

Preferred ADAM{dis} proteins may be identified on the basis of their ability to mediate interactions between myeloma cells, stromal cells and endothelial cells using known technology (see e.g. Myles et al (1994) PNAS USA Vol. 91, pages 4195-4198, the contents of which are incorporated herein by reference). This involves the preparation of peptides (e.g. synthetic peptides) containing the disintegrin-like domain of the ADAM coupled to covaspheres, which are then incubated with different cell types. Fluorescence microscopy is then used to detect binding of the covaspheres to cells thereby implicating these peptides and hence the ADAM{dis} in cell/cell interactions.

Therapeutically active ADAM{dis} domains may also be identified by testing for the ability to inhibit myeloma cell/ECM substrate adhesion using quantitative adhesion assays. Myeloma cells adhere to a number of substrates including type I and IV collagen, and fibronectin when coated on 96 well plates. Adhesion of myeloma cells to these substrates can be quantitated by staining cells with propidium iodide and measuring nuclear fluorescence using a fluorescence plate reader. Synthetic peptides corresponding to the ADAM{dis} can be used in these assays to test for activity in the disruption of myeloma cell/ECM adhesion.

Myeloma cells are known to adhere to a number of cells including stromal cells, bone cells and possibly endothelial cells. These activities have now been discovered to be mediated by the ADAM proteins of the invention. Peptides or other compounds which inhibit adhesion can be identified by adding them to co-cultures of stromal and myeloma cells and assessing their ability to inhibit adhesion by staining with FITC labelled anti-immunoglobulin light chain antibodies and quantitated by both fluorescence microscopy and fluorescence plate reader.

The present invention also relates to a method of diagnosis comprising the step of detecting the expression of an ADAM gene in a selected class of cells. Preferably, the diagnostic method of the invention is a method of diagnosing multiple myeloma comprising the step of detecting the expression of ADAM 12 in myeloma cells.

Expression of the ADAM genes in such diagnostic methods is preferably detected and/or measured by dual fluorescence in situ hybridization (FISH), according to the method described in Sati et al. (1996) (the content of which is included herein by reference). This technique comprises the steps of preparing cyto-spins of bone marrow and peripheral blood from the patient, followed by fixation, FISH processing and hybridization. The hybridization may be

carried out with two probes. One probe is a cocktail of directly fluorescent oligonucleotides specific for the immunoglobulin light chain (enabling the detection of malignant plasma cells in a mixed cell population), and the other probe is specific for the ADAM gene labelled with digoxygenin and detected with anti-DIG antibody conjugated with rhodamine.

Preferably, the isolated protein consists essentially of: (a) ADAM{pro}; or (b) ADAM{met}; or (c) ADAM{pro} and ADAM{met}; or (d) ADAM{pro}, ADAM{met} and ADAM{dis}; or (e) ADAM{met} and ADAM{dis}; or (f) ADAM{dis}; or (g) ADAM{egf}; or (h) ADAM{met} and ADAM{egf}; or (i) ADAM{met}, ADAM{dis} and ADAM{egf}; or (j) ADAM{pro}, ADAM{met}, ADAM{dis} and ADAM{egf}, (k) ADAM{dis} and ADAM{fus}, (l) ADAM{met}, ADAM{dis} and ADAM{fus}, and may form part of a pharmaceutical composition or for use in therapy, diagnosis or prophylaxis.

Some or all of the above ADAM domain combinations are functionally complementary in that the conjunction of the selected domains promotes or enables a functional interaction therebetween, as explained above in the case of the ADAM 12 domain combinations.

In each of the specific embodiments of the invention which

incorporate an ADAM{fus} domain (for example the ADAM 12{fus} domain), the ADAM{fus} domain preferably further comprises (e.g. occurs in the amino acid sequence context of) the cysteine repeat pattern Cys-5/6X-Cys-4X-Cys-14X-Cys-12/13X-Cys-9/10/11X-Cys-6X-Cys-4X-Cys, or a functionally equivalent repeat pattern.

According to another aspect of the present invention, there is provided a pharmaceutical composition comprising or consisting essentially of an inhibitor of an ADAM{met}. This composition may: (a) comprise or consist essentially of an ADAM{pro} (for example the ADAM{pro} cysteine switch); or (b) comprise or consist essentially of an ADAM{egf}; or (c) be an antibody to an ADAM{met}; or (d) be a pseudosubstrate of an ADAM{met}; or(e) bind to and block an ADAM{met} effector site; or (f) be a reprolysin inhibitor or derivative thereof; or comprise or consist essentially of the amino acid sequence motif PXXCGV, or a functional equivalent thereof; or (h) comprise or consist essentially of the amino acid sequence motif PLKCGV, or a functional equivalent thereof; or (i) be a peptide-hydroxamate; or (j) be a pyroglutamate peptide; or (k) be oprin, a member of the oprin family or a homologue, derivative, mutein or equivalent thereof; or (l) be an alpha 1B glycoprotein or a homologue, derivative, mutein or equivalent thereof; or (m) be a macroglobulin (e.g. alpha

2 macroglobulin) or a homologue, derivative, mutein or equivalent thereof; or (n) ligand the active site Zn ion of the ADAM{met}; or (o) be a high-cysteine subdomain of an cysteine rich region.

The inhibitor of the ADAM{met} domain is preferably an inhibitor of the ADAM 12{met} domain. Particularly preferred are inhibitors of the protease activity of the ADAM{met} domain. However, the inhibitor can also be an inhibitor of other activities of the ADAM{met}, for example its effector binding activity.

For example, the inhibitor may block the ability of the ADAM{met} domain to act as a sink for various molecules, for example effector molecules or substrates (for example effector molecules and/or substrates for other enzymes).

The invention also relates to a pharmaceutical composition comprising or consisting essentially of an inhibitor (for example an agonist or antagonist) of an ADAM{dis}. Such a composition is preferably one which: (a) is an ADAM{dis} receptor mimetic (e.g. an integrin mimetic); (b) is an ADAM{dis} receptor binding sequence mimetic (e.g. a peptide (e.g. a trimer, tetramer or pentamer) comprising or consisting essentially of the amino acid sequence ECD and/or TDE); or (c) is an antibody to an ADAM{dis}.

Other ADAM{dis} receptor binding sequence mimetics include peptides comprising or consisting essentially of the amino acid sequences TSE and/or ESE and/or VGP and/or EDE and/or VNE and/or KDK.

Particularly preferred are compositions which comprise or consist essentially of an ADAM{dis} receptor binding sequence mimetic wherein the ECD and/or TDE sequence is within a rigid disulfide loop, a cyclic peptide or associated with an ADAM{fus}, for example an ADAM{fus} which further comprises the cysteine repeat pattern Cys-5/6X-Cys-4X-Cys-14X-Cys-12/13X-Cys-9/10/11X-Cys-6X-Cys-4X-Cys, or a functionally equivalent repeat pattern.

In all aspects of the invention relating to an ADAM protein, the ADAM is preferably ADAM 12, as hereinbefore defined. Particularly preferred in this respect are the *h*ADAM 12 and *b*ADAM 12 proteins. In all aspects of the invention relating to the ADAM{fus} domain, the domain preferably further comprises the cysteine repeat pattern Cys-5/6X-Cys-4X-Cys-14X-Cys-12/13X-Cys-9/10/11X-Cys-6X-Cys-4X-Cys, or a functionally equivalent repeat pattern.

In another aspect, the invention also relates to an isolated ADAM interdomainase. The term interdomainase is used herein to define a protease which cleaves (preferably

specifically) an ADAM protein within a junction region between two domains thereof. The junction region may define an interdomainase-specific target site.

Such interdomainases can be identified and purified for example using techniques known to those skilled in the art on the basis of their interactions with the ADAM proteins of the invention. For example, they can be identified and/or isolated by affinity chromatography and/or chemical crosslinking.

The interdomainase of the invention preferably cleaves at any or all of the: (a) ADAM{pro}-ADAM{met} interdomain boundary; or (b) ADAM{met}-ADAM{dis} interdomain boundary; or (c) ADAM{dis}-ADAM{fus} interdomain boundary; or (d) ADAM{fus}-ADAM{egf} interdomain boundary; or (e) ADAM{egf}-ADAM{tmt} interdomain boundary.

The invention also contemplates inhibitors of the interdomainase of the invention. Such inhibitors are preferably peptides (e.g. peptides comprising a tetra and/or dibasic residue) consisting essentially of an interdomain amino acid sequence selected from: (a) an ADAM{pro}-ADAM{met} interdomain amino acid sequence; or (b) an ADAM{met}-ADAM{dis} interdomain amino acid sequence; or (c) an ADAM{dis}-ADAM{fus} interdomain amino acid sequence;

or (d) an ADAM{fus}-ADAM{egf} interdomain amino acid sequence; or (e) an ADAM{egf}-ADAM{tmt} interdomain amino acid sequence, or functionally equivalent amino acid sequences derived therefrom or modelled thereon. Such sequences act as interdomain boundary sequence mimetics, and act as interdomainase decoys in vivo.

The interdomainase or inhibitor of the invention is preferably for use in therapy, diagnosis or prophylaxis or provided in the form of a pharmaceutical excipient, a unit dosage form or in a form suitable for local or systemic administration. Conveniently, the interdomainase or inhibitor of the invention is provided as a pharmaceutical composition.

The invention also contemplates isolated nucleic acid (for example RNA (e.g. mRNA) or DNA (e.g. cDNA) encoding the ADAM protein or interdomainase of the invention, as well as nucleic acid probes (e.g. labelled nucleic acid probes) which are selectively hybridizable therewith. Such probes are preferably single stranded DNA or RNA probes.

As used herein and applied to nucleic acid (for example, DNA, cDNA or RNA), the term isolated indicates that the nucleic acid exists in a physical milieu distinct from that in which it occurs in nature. For example, the nucleic

acid may be substantially isolated with respect to the complex cellular milieu in which it naturally occurs, or simply present in a different nucleic acid sequence context from that in which it occurs in nature (for example, when cloned in a vector, in the form of a restriction fragment or present in a heterologous host cell). Thus, the nucleic acid (e.g. DNA) of the invention may be isolated in the sense used herein, yet present in any of a wide variety of vectors and in any of a wide variety of host cells (or other milieu, such as buffers, viruses or cellular extracts).

The DNA of the present invention embraces DNA having any sequence so long as it encodes the proteins of the invention. As a result of degeneracy in the genetic code, any particular amino acid sequence of the invention may be encoded by many different DNA sequences.

The invention also contemplates vectors (e.g. recombinant vectors) comprising the nucleic acid of the invention. The nature of the vector is not critical to the invention. Any suitable vector may be used, including plasmid, virus, bacteriophage, transposon, minichromosome, liposome or mechanical carrier.

The invention also embraces recombinant expression vectors,

which vectors are defined herein as DNA construct used to express DNA which encodes a desired protein and which includes a transcriptional subunit comprising an assembly of: 1) genetic elements having a regulatory role in gene expression, for example, promoters and/or enhancers; 2) a structural or coding sequence which is transcribed into MRNA and translated into protein, and 3) appropriate transcription and translation, initiation and termination sequences.

Using methodology well known in the art, recombinant expression vectors of the present invention can be constructed. Possible vectors for use in the present invention include, but are not limited to: for mammalian cells, pJT4 (discussed further below), pcDNA-1 (Invitrogen, San Diego, CA) and pSV-SPORT 1 (Gibco-BRL, Gaithersburg, MD0; for insect cells, pBlueBac III or pBlueBacHis baculovirus vectors (Invitrogen, San Diego, CA); and for bacterial cells, PET-3 (Novagen, Madison, WI). The DNA sequence coding for the protein of the invention (e.g. an ADAM protein or an interdomainase) can be present in the vector operably linked to regulatory elements.

Particularly preferred for bacterial expression systems are the polymerase T7 based vectors in suitable E. coli hosts (e.g. BL21, BL21, DE3 or similar strains containing plasmid

pLysS or pLysE to control expression). Particularly preferred for eukaryotic gene expression is the vector pcDNA3 (Invitrogen) in which transcription is under the control of the human cytomegalovirus promoter). This vector can be transformed into NSO mouse myeloma cells (with e.g. vectors having strong eukaryotic primers such as pcdna3 (Invitrogen)) according to the protocols described in e.g. Murphy and Willenbrock (1995) Methods in Enzymology Vol. 248, pages 496-510, the content of which is incorporated herein by reference.

If secretion into the medium is required, the ADAM{tmt} domain is preferably removed, e.g. by site-directed mutagenesis.

The vector may preferably comprise an expression element or elements operably linked to the DNA of the invention to provide for expression thereof at suitable levels. Any of a wide variety of expression elements may be used, and the expression element or elements may for example be selected from promoters, enhancers, ribosome binding sites, operators and activating sequences. Such expression elements may be regulatable, for example inducible (via the addition of an inducer).

As used herein, operably linked refers to a condition in

which portions of a linear DNA sequence are capable of influencing the activity of other portions of the same linear DNA sequence. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous in reading frame.

Conveniently, the vector of the invention is a viral vector, being for example based on simian virus 40, adenoviruses (e.g. human adenoviruses), retroviruses, and papillomavirus.

The vector may further comprise a positive selectable marker and/or a negative selectable marker. The use of a positive selectable marker facilitates the selection and/or identification of cells containing the vector.

Also contemplated by the invention is a host cell comprising one or more vectors of the invention. Any suitable host cell may be used, including prokaryotic host

cells (such as Escherichia coli and Bacillus subtilis) and eukaryotic host cells (such as mammalian cells and yeast cells). Host cells may be stably transfected or transiently transfected within a recombinant expression plasmid or infected by a recombinant virus vector. Other host cells include permanent cell lines derived from insects such as Sf-9 and Sf-21, and permanent mammalian cell lines such as Chines hamster ovary (CHO) and SV40-transformed African green monkey kidney cells (COS). COS cells are particularly preferred for transient expression.

In another aspect, the invention also contemplates a process for producing the protein or interdomainase of the invention comprising the steps of: (a) culturing the host cell of the invention, and (b) purifying the protein or interdomainase from the cultured host cells (e.g. from a culture supernatant or cell fraction).

In another aspect, the invention relates to a process for producing the protein or interdomainase of the invention comprising the steps of: (a) probing a gene library with a nucleic acid probe which is selectively hybridizable with nucleic acid encoding the protein or interdomainase (for example having a sequence which is comprised in a gene corresponding to the sequence shown in Fig. 1), to produce

a signal which identifies a gene that selectively hybridizes to the probe; and (b) expressing the gene identified in step (a) (for example by cloning into a host cell) to produce the protein.

Refolding, dimerization, trimerization, tetramerization and/or oxidation (e.g. in vitro or in vivo) to establish inter- and/or intra-molecular disulphide bonds may be necessary to obtain fully active ADAM protein (or active domains thereof). This can be achieved using standard techniques known to those skilled in the art.

Such recombinantly-produced ADAM protein (recombinant ADAM protein) can be produced relatively inexpensively in large quantities, and can be relatively easily purified.

As used herein, the term selectively or specifically hybridizable indicates that the sequence of the probe is such that binding to a unique (or small class) of target sequences can be obtained under more or less stringent hybridization conditions. This method of the invention is not dependent on any particular hybridization conditions, which can readily be determined by the skilled worker (e.g. by routine trial and error or on the basis of thermodynamic considerations).

The invention also embraces proteins obtainable by the above-described methods, as well as proteins obtained by the above-described methods.

Also contemplated by the invention are vectors (e.g. retroviral vectors) for altering the expression of an ADAM (e.g. ADAM 12) in vivo comprising the DNAs of the invention and/or upstream or downstream DNA. Such vectors are preferably formulated as pharmaceutical compositions.

In another aspect, the invention relates to a method for screening for therapeutically active drugs comprising the steps of: (a) contacting a candidate drug with the ADAM protein or interdomainase of the invention, and (b) determining whether binding between the candidate drug and protein/interdomainase occurs, wherein binding is indicative of a therapeutically active drug.

The method described above is useful for screening large numbers of drug candidates for therapeutic activity. Preferably, the method is employed in high throughput screening of drug candidates. Drug candidates identified by the method of the invention can be further modified or used directly as therapeutic drug candidates to activate or inhibit the natural functions of the protein in vivo.

In another aspect, the invention relates to a method for evaluating the therapeutic activity of a drug comprising the steps of: (a) contacting a candidate drug with the ADAM protein or the interdomainase of the invention, and (b) measuring the binding affinity of the candidate drug for the protein or interdomainase.

Preferably, the protein used in the screening and evaluation methods of the invention comprises an ADAM{met} (e.g. ADAM 12{met}) and the drug evaluated is preferably an inhibitor of an ADAM{met} domain.

The invention also relates to a method for synthesizing a therapeutically active drug comprising the steps of: (a) generating a three-dimensional model of the active site (e.g. the catalytic or ligand binding site) of the ADAM protein or interdomainase of the invention (e.g. by computer analysis of its amino-acid sequence and/or by X ray crystallography of the protein/interdomainase or fragment thereof), and (b) modelling the drug with reference to the three-dimensional model generated in step (a), the drug for example being an inhibitor of an ADAM{met} and the protein comprising an ADAM{met} (e.g. ADAM 12{met}).

Many different techniques exist for generated a three-

dimensional model for use in the above-described methods, and all are suitable for use in the method of the invention. Conveniently, the three-dimensional model is generated by computer analysis of the amino-acid sequence of all or a portion of the proteins (or individual domains) of the invention (for example the ADAM{met} domain). Alternatively, the three-dimensional model could be generated by X ray crystallography of the protein (or domains, fragments or derivatives thereof), or by NMR techniques. These techniques could also be applied to the protein-substrate/effector complex, the results of which could also be used as the basis for the rational design of therapeutic agents.

Conveniently, a three-dimensional model can be generated on the basis of comparisons with the related or similar reprolysin crystal structures which are known in the art.

The invention also embraces therapeutically active drugs which have been screened, evaluated or synthesised by the methods of the invention.

In another aspect, the invention relates to an antibody (e.g. a monoclonal antibody) which binds (preferably specifically) to the ADAM protein or interdomainase of the invention.

In another aspect, the invention relates to a test kit comprising the protein or interdomainase of the invention, for example for use in the methods of the invention. Preferably in such test kits: (i) the protein is bound to a solid support and/or (ii) the kit further comprises a labelled (e.g. radiolabelled or fluorescently labelled) protein substrate, receptor or ligand and/or (iii) the kit further comprises the antibody of the invention.

The invention also relates to a nucleic acid probe which is selectively hybridizable with the DNA of the invention. Again, the term selectively hybridizable indicates that the sequence of the probe is such that binding to a unique (or small class) of target sequences can be obtained under more or less stringent hybridization conditions.

Preferably, the nucleic acid probe is selectively hybridizable with (for example having a sequence which is comprised in) a gene corresponding to that shown in Fig. 2.

The protein of the invention may be labelled, for example with a flourescent label, a radioisotope or an enzyme. Such labelled proteins may be particularly suitable for use in the test kits of the invention.

Also contemplated by the present invention are test kits

comprising the protein of the invention. Such kits are useful, for example, in the screening and evaluating methods of the invention. The protein comprised in the kits of the invention are preferably bound to a solid support and may conveniently include a labelled (e.g. radioactively-labelled) substrate (for example for use in competitive binding assays or in displacement assays).

Also contemplated by the invention are antibodies which bind to the protein of the invention. Such antibodies can be prepared by employing standard techniques well known to those skilled in the art, using any of the proteins of the invention or individual domains/fragments thereof as antigens for antibody production. These antibodies can be employed for diagnostic applications, therapeutic applications, and the like. Preferably for therapeutic applications, the antibodies are monoclonal antibodies.

The antibodies of the invention may advantageously bind specifically to the proteins of the invention. Antibodies specific for the catalytic or effector sites of the various domains of the proteins of the invention may act as substrate/effector mimetics. Specific binding may be exploited in imaging techniques, for example to assess the extent to which the ADAM proteins are active or their distribution in vivo. They may also be used to identify and

isolate further embodiments of the proteins of the invention.

The invention also contemplates antibody derivatives, including antibody fragments (e.g. Fab fragments), chimaeric antibodies (including humanized antibodies) and antibody derivatives (such as fusion derivatives comprising an antibody-derived variable region and a non-immunoglobulin peptide having for example enzyme or conjugative activity).

The invention also contemplated mimetics (for example, the antibodies of the invention described above), agonists and antagonists of the proteins of the invention.

Medical uses

The medicaments, pharmaceutical compositions and drugs of the invention (which include the subject matter of the claims, inter alia the ADAM proteins, domains thereof, homologues thereof, inhibitors thereof, pharmaceutical compositions based thereon, interdomainases, interdomainase inhibitors, nucleic acids, vectors, host cells, test kits and antibodies) finds application in various forms of therapy, prophylaxis and diagnosis.

These medicaments, pharmaceutical compositions and drugs can be administered in a clinical setting by intraperitoneal, intramuscular, intravenous, or subcutaneous injection, implant or transdermal modes of administration, and the like.

In preferred embodiments, the invention finds application in the therapy, prophylaxis and/or diagnosis of disorders or diseases of connective tissue, disorders or diseases of the skeletal system (e.g. bone), inflammatory diseases or disorders (for example asthma), cancer (e.g. multiple myeloma), coagulopathies (e.g. thrombo-embolic disorders), haemorrhagic disorders or diseases and amyloidosis (e.g. Alzheimer's disease).

(a) Disorders or diseases of connective tissue

The term connective tissue is used herein in a broad sense to embrace collagenous (including cartilaginous) tissues.

Cartilage consists of chondrocytes surrounded by an extracellular matrix. The matrix is a mesh of type II collagen fibrils in which proteoglycan aggregates (aggrecan) are trapped. Damage to one or both of these molecules results in major changes in cartilage function.

In the arthritides (for example rheumatoid arthritis and osteoarthritis), a major feature of the pathology is the loss of joint function due to erosion of articular cartilage. Destruction of collagen and aggrecan is mediated by proteolytic enzymes produced by either the chondrocytes themselves (in osteoarthritis) or by cells which have infiltrated the joint during the disease process (rheumatoid arthritis).

Aggrecan degradation is mediated by an aggrecanase, which produces a characteristic cleavage between E^{373} and A^{374} of the aggrecan molecule. The cleavage sites are shown below:

known aggrecanase cleavage sites	1	TEGE.ARGS
	2	VSQE.LGQR
	3	TAQE.AGEG
*h*ADAM 12 and *b*ADAM 12 cleavage site. Releases ADAM{met} and ADAM{dis}.		MLQE.AVRQ

The present inventors have now determined that certain members of the ADAM family (including ADAMs 10, 11 and 12) exhibit aggrecanase activity, are expressed in chondrocytes and are responsible for destruction of aggrecan in e.g.

arthritides and emphysema (especially atrophic emphysema and inherited emphysema).

Accordingly, in preferred embodiments of the invention the disorder or disease treated, diagnosed or prevented is: (a) that requiring regulation of aggrecan degradation (e.g. the regulation of aggrecanase activity); or (b) that requiring the regulation of cartilage breakdown; or (c) that requiring the prevention or reduction of cartilage breakdown; or (d) arthritides, for example osteoarthritis or rheumatoid arthritis.

Other connective tissue disorders which may be treated or prevented by the drugs of the invention include giant-cell arteritis, polyarteritis nodosa, polymyalgia rheumatica, polymyositis and systemic lupus erythematosus.

Drugs (e.g. inhibitors and interdomainases/interdomainase inhibitors as hereinbefore described) based on ADAM 10, ADAM 11 (MDC) and ADAM12 are particularly preferred in the treatment or prophylaxis of disorders or diseases of connective tissue.

(b) **Skeletal diseases and disorders**

Particularly preferred is the treatment, prophylaxis or

diagnosis of diseases characterized by bone resorption, osteoporosis (for example postmenopausal osteoporosis), Paget's disease, metastatic bone disease or myeloma associated bone disease.

The ADAM{met} domain is involved in the degradation of the bone matrix, particularly of the unmineralized matrix that protects bone from uncontrolled resorption. Thus, inhibitors of the ADAM{met} domain may be used to prevent degradation of the bone matrix and/or the unmineralized matrix and so reduce bone resorption. Thus, ADAM{met} inhibitors (or those of the cognate interdomainase(s) responsible for activating ADAM{met}) form the basis of a new class of anti-resorptive drugs.

The ADAM{dis} domain mediates adhesion of osteoclasts and osteoblasts to bone and to the ECM. Thus, inhibitors of the ADAM{dis} domain may be used to prevent adhesion of osteoclasts and osteoblasts to bone and to the ECM. Thus, ADAM{dis} inhibitors (or those of the cognate interdomainase(s) responsible for activating ADAM{dis}) form the basis of a new class of anti-resorptive drugs.

The ADAM{dis} domain is involved in the prevention of adhesion of osteoclasts and osteoblasts to bone and the ECM. Thus, ADAM{dis} or ADAM{dis} activators (such as the

cognate interdomainase(s) responsible for activating ADAM{dis}) form the basis of a new class of anti-resorptive drugs.

The ADAM{fus} domain is involved in the fusion of osteoclast precursors to form multinucleated osteoclasts. Thus inhibitors of the ADAM{fus} domain may be used to prevent osteoclast fusion and so reduce osteoclast-mediated bone resorption. Thus, ADAM{fus} inhibitors, inhibitors or activators of the interdomainase(s) responsible for activating (e.g. releasing from a membrane) ADAM{fus} or the interdomainases per se form the basis of a new class of anti-resorptive drugs.

The ADAM{fus} domain is also involved in myoblast fusion to form the muscular syncytium. Thus, ADAM{fus} inhibitors, inhibitors or activators of the interdomainase(s) responsible for activating (e.g. releasing from a membrane) ADAM{fus} or the interdomainases per se may be used to regulate muscle development, e.g. in the treatment or prophylaxis of various myasthenias (including inflammatory muscle disorders such as myopathies and vascular muscle disorders such as polymyositis).

The ADAM{fus} domain is also involved in the fusion of giant cells. Thus, ADAM{fus} inhibitors, inhibitors or

activators of the interdomainase(s) responsible for activating (e.g. releasing from a membrane) ADAM{fus} or the interdomainases per se may be used to regulate giant cell development e.g. in the treatment or prophylaxis of giant-cell arteritis.

The ADAM{fus} domain is also involved in neuronal development. Thus, ADAM{fus} inhibitors, inhibitors or activators of the interdomainase(s) responsible for activating (e.g. releasing from a membrane) ADAM{fus} or the interdomainases per se may be used to regulate neuronal development.

The ADAM{fus} domain is also involved in fertility, including implantation in utero. Thus, ADAM{fus} inhibitors, inhibitors or activators of the interdomainase(s) responsible for activating (e.g. releasing from a membrane) ADAM{fus} or the interdomainases per se also find application in fertility treatment, contraception and abortion.

(c) Inflammatory diseases and disorders

The inflammatory diseases with which the present invention is concerned may be characterized by cell infiltration and the secretion of proinflammatory cytokines (for example,

rheumatoid arthritis is characterized by the entry of macrophages into the joint space).

Proinflammatory cytokines include interleukin 1ß (IL-1ß) and tumour necrosis factor α (TNFα). Both of these cytokines are synthesised as precursors and are processed by proteinases to give mature soluble form by proteolytic shedding mechanisms (explained in more detail below). For example, IL-1ß is processed by a cysteine proteinase known as ICE (IL-1ß converting enzyme). Soluble forms of the TNFα receptor are also processed by proteinases.

The invention finds particular application in relation to inflammatory and/or allergic disorders or diseases, for example those mediated by proinflammatory cytokines and/or by cell (e.g. macrophage) infiltration. For example, the invention finds application in the treatment or prophylaxis of inflammation attendant on infection, trauma, graft rejection, autoimmune disorders, psoriasis and diseases of the peripheral and central nervous sytem. The invention may also be used in the treatment or prophylaxis of scleroderma (preferably together with adjunctive therapy) and other skin diseases.

Particularly preferred is the treatment or prophylaxis of rheumatoid arthritis and asthma.

Drugs (e.g. those defined in the claims including inter alia inhibitors and interdomainases/interdomainase inhibitors as hereinbefore described) based on ADAM 8 are particularly preferred in the treatment or prophylaxis of inflammatory disorders or diseases.

In preferred embodiments, the drugs of the invention are used in the treatment or prophylaxis of allergic disorders, inflammatory diseases, systemic tissue damage and/or apoptosis by administration in an amount sufficient to regulate, promote or reduce membrane protein shedding (which process is described in more detail below).

(d) Cancer

The drugs of the present invention may be used in the treatment or prophylaxis of cancer.

Preferred modes of cancer therapy, prophylaxis or diagnosis according to the invention include the detection or prevention of tumour progression or the detection or prevention of tumour metastasis.

In various cancers, tumour cells undergo uncontrolled proliferation which may be stimulated by local growth factors and cytokines. For example, myeloma cells grow in

response to interleukin-6 (IL-6), which binds the IL-6 receptor to produce a signal which is transduced through gp130. The soluble form of IL-6 receptor appears unique in that it binds IL-6 but still retains the capacity to stimulate signal transduction thereby acting as an agonist (rather that an antagonist, as is the case with other soluble receptors) of its cognate cytokine. The soluble IL-6R is shed from cell membranes by proteolytic cleavage from the cell surface, and the ADAM proteins of the invention are involved in this (and other) shedding processes.

Thus, in a preferred embodiment, the drugs of the invention may be used to inhibit or prevent tumour progression by inhibiting cytokine-mediated tumour stimulation at the level of proteolytic shedding of membrane bound cytokines or receptors (e.g. membrane-bound IL-6 receptor).

The invention also finds application in the treatment or prophylaxis of metastatic disease which characterizes the terminal stages of most malignancies. Tumour metastasis involves loss of adhesion with and shedding from the primary tumour, degradation of the basement membrane components and migration into the blood supply.

The ADAM{met} domain promotes metastasis by assisting basement membrane cleavage and so promoting tumour cell

extravasation. Thus inhibitors of ADAM{met} (or inhibitors of the ADAM{met}-releasing interdomainase(s)) may be used to prevent or reduce basement membrane cleavage and so reduce or eliminate metastasis. Thus, ADAM{met} inhibitors (or those of the cognate interdomainase(s) responsible for activating ADAM{met}) may form the basis of a new class of anticancer drugs.

Particularly preferred is the treatment or prophylaxis of ovarian metastasis, choriocarcinoma and trophoblast tumours.

The ADAM{dis} domain is involved in the loss of cell-cell and cell-matrix adhesion necessary for the metastasis of solid tumours. Thus inhibitors of ADAM{dis} may be used to promote or maintain cell-cell and/or cell-matrix adhesion and so reduce or eliminate metastasis. Thus, ADAM{dis} inhibitors (or those of the cognate interdomainase(s) responsible for activating ADAM{dis}) may form the basis of a new class of anticancer drugs.

The ADAM{dis} domain is involved in cell-cell and cell-matrix adhesion necessary to prevent metastasis of solid tumours. Thus ADAM{dis} or ADAM{dis} activators (such as the cognate interdomainase(s) responsible for activating ADAM{dis}) may be used to maintain cell-cell and/or cell-

matrix adhesion and so reduce or eliminate metastasis.

Particularly preferred according to the invention is the treatment, prophylaxis or diagnosis of multiple myeloma. This is a B cell neoplasm resulting in clonal expansion of the plasma cell compartment. Myeloma cells are found in the bone marrow where they may represent as many as 90% of the mononuclear cells. They interact with cells of the bone marrow microenvironment, disrupting normal haemopoesis and causing neutropenia, anaemia, bone destruction and hypercalcemia. In the terminal stages of the disease, malignant plasma cells exit the bone marrow and are found in the peripheral blood in a disease known as plasma cell leukaemia.

While chemotherapy increases life expectancy, median survival remains low (36 months), making the understanding of the pathophysiology of multiple myeloma and the identification of new approaches to the management of the disease a priority.

Recent studies have shown that the stroma of patients with multiple myeloma differ from that of normal subjects in that there is a reduction in the amount and a simpler organization of the extracellular matrix and altered expression of adhesion molecules. Under normal

circumstances the ECM within the bone marrow microenvironment is being constantly remodelled, a process widely believed to be performed by members of the matrix metalloproteinase family of enzymes and regulated by a family of naturally occurring inhibitors, the tissue inhibitors of metalloproteinases (TIMPs).

The present inventors have now found that certain members of the ADAM family (including ADAM 12) are involved in changes in cell/cell and/or cell/matrix interactions associated with progression to plasma-cell leukaemia, while the ADAM{met} proteins are involved in the degradation of the extracellular matrix observed in multiple myeloma.

The ADAM{dis} domain is involved in the loss of cell-cell and cell-matrix adhesion necessary for the progression to plasma-cell leukaemia. Thus inhibitors of ADAM{dis} may be used to promote or maintain cell-cell and/or cell-matrix adhesion and so reduce or eliminate progression to plasma-cell leukaemia. Thus, ADAM{dis} inhibitors (or those of the cognate interdomainase(s) responsible for activating ADAM{dis}) may form the basis of a new class of drugs for the treatment or prophylaxis of multiple myeloma.

The ADAM{dis} domain is involved in cell-cell and cell-matrix adhesion necessary to prevent progression to plasma-

cell leukaemia. Thus ADAM{dis} or ADAM{dis} activators (such as the cognate interdomainase(s) responsible for activating ADAM{dis}) may be used to maintain cell-cell and/or cell-matrix adhesion and so reduce or eliminate progression to plasma-cell leukaemia.

The ADAM{met} domain promotes degradation of the extracellular matrix in multiple myeloma. Thus inhibitors of ADAM{met} may be used to prevent or reduce degradation of the matrix. Thus, ADAM{met} inhibitors (or those of the cognate interdomainase(s) responsible for activating ADAM{met}) may form the basis of a new class of drugs for the treatment or prophylaxis of multiple myeloma.

Drugs (e.g. inhibitors and interdomainases/interdomainase inhibitors as hereinbefore described) based on ADAM 11 (MDC) and ADAM12 are particularly preferred in the treatment or prophylaxis of cancer.

(e) Coagulopathies

The drugs of the present invention may be used in the treatment or prophylaxis of various coagulopathies. For example, the ADAM{met} or the appropriate ADAM{met}-releasing interdomainases may be used to interfere with coagulation. This may be applied in the treatment or

prophylaxis of disseminated intravascular coagulation (DIC) or in other diseases associated with intravascular coagulation, such as meningococcal meningitis.

The ADAM{met} or the appropriate ADAM{met}-releasing interdomainases may also be used as fibrinolytic agents, for example to disperse blood clots (e.g. in the treatment or prophylaxis of thrombosis).

The ADAM{dis} or the appropriate ADAM{dis}-releasing interdomainases may also be used to promote clotting, e.g. in the treatment or prophylaxis of haemophilia or thrombocytopenia.

The ADAM{dis} or the appropriate ADAM{dis}-releasing interdomainases may also be used to prevent platelet aggregation, which can lead to occlusive platelet plugs. This finds particular application in the manufacture of vascular grafts, the luminal surface of which can be coated with the ADAM{dis} of the invention to prevent platelet aggregation thereon.

(f) Amyloidosis

As used herein, the term "amyloidosis" relates to a collection of etiologically unrelated diseases

characterized by protein deposition in tissues arising as the end result of aberrant protein processing. Alzheimer's disease is one important example of such a disease. An aberrant proteolytic cleavage event is thought to be associated with amyloidosis in Alzheimer's, and the ADAM{met} inhibitors of the present invention may be used to reduce or prevent this aberrant processing.

(g) Control of development and inter- and intracellular signalling

In another aspect, the invention relates to methods of regulating, promoting or reducing a variety of biochemical processes. Such methods may be used to control or treat a number of different diseases or disorders.

Thus, in another aspect the invention relates to methods of regulating, promoting or reducing: (a) membrane protein shedding or processing; (b) ADAM processing; (c) cell-cell adhesion, contact or communication (e.g. juxtacrine stimulation, as described by Massague (1990) J. Biol. Chem. Vol. 265, pages 21393-21396); (d) aggrecan degradation, e.g. via the regulation, promotion or reduction of aggrecanase activity; (e) the degradation of basement membrane components (e.g. type IV collagen and/or fibronectin); (f) osteoclast precursor fusion, and (g)

collagen type II degradation and/or removal.

Many proteins exist as membrane-bound and soluble isoforms and the function of the protein may differ dramatically according to its state. One important mechanism for the generation of a soluble isoform from a membrane-bound precursor is proteolytic cleavage, which results in "shedding" of the soluble isoform from the membrane. This mechanism can be used to rapidly change the phenotype of a cell, to simultaneously remove a surface protein while creating a soluble isoform (there being a functional synergy between these two events) and to meet a need for a rapid switch from a local to a systemic distribution of a given protein (such as a cytokine).

The present inventors have now discovered that the ADAM proteins of the invention are involved in the proteolytic shedding of membrane proteins. This discovery can be exploited in the design of drugs which regulate, promote or reduce proteolytic shedding in the treatment, diagnosis and prophylaxis of a wide range of different diseases and disorders which arise from dysfunctions in membrane protein shedding dynamics.

In such applications the membrane protein may be a receptor, a receptor ligand, a cell adhesion molecule, a

leukocyte antigen or an ectoenzyme.

Particularly preferred receptors are cytokine receptors, for example a receptor for any one of TNF, IL-6, CSF-1, NGF, EGF and GH. Other preferred receptors include those for transferrin, poly-Ig and folate.

Particularly preferred receptor ligands are cytokines (e.g. lymphokines or monokines) or growth factors, for example any one of Steel factor, TNF (e.g. TNFα), TGF-α, CSF-1 Fas ligand (FasL), or *c-kit* ligand.

Thus, the invention finds application in the treatment or prophylaxis of fever, shock (e.g. septic and endotoxic shock), cachexia and cerebral malaria.

The Fas ligand is a member of the tumour necrosis family. It induces apoptosis in Fas-bearing cells. The membrane-bound FasL is converted into the soluble form (sFasL) by ADAM{met}. Patients with large granular lymphocytic (LGL) leukemia and natural killer (NK) cell lymphoma have elevated levels of sFasL in serum, resulting in systemic tissue damage (Tanaka et al. (1996) Nature Medicine, Vol. 2(3), pages 317-322). Thus, ADAM{met} inhibitors or the appropriate ADAM{met}-releasing interdomainase inhibitors may be used to regulate (e.g. reduce or eliminate) sFasL

production in vivo, for example in the treatment or prophylaxis of leukemia and lymphoma (and particularly in the treatment or prophylaxis of the systemic tissue damage associated with these diseases). The ADAM{met} inhibitors or the appropriate ADAM{met}-releasing interdomainase inhibitors of the invention may also be used in the treatment or prophylaxis of patients having high levels of sFasL in their serum (e.g. neutropenic or hepatocompromized individuals). Thus, the ADAM{met} inhibitors or the appropriate ADAM{met}-releasing interdomainase inhibitors of the invention find application in the treatment or prophylaxis of various diseases that are accompanied with systemic tissue destruction or apoptosis.

AIDS is associated with apoptosis of $CD4^+$ T cells and small amounts of sFasL continuously kill $CD4^+$ T cells. Thus, the ADAM{met} inhibitors or the appropriate ADAM{met}-releasing interdomainase inhibitors of the invention may also find application in the treatment or prophylaxis of AIDS.

Particularly preferred cell adhesion molecules include gp 100, Leu 8, ELAM-1, GMP-140, glycoprotein Ib and neural cell adhesion molecule (NCAM).

Particularly preferred leukocyte antigens include immunoglobulin, CD8, class I MHC, IL 2 receptor, IL 4

receptor, CD16, CD23 and CD14.

Particularly preferred ectoenzymes include angiotensin-converting enzyme (ACE), NEP 24.11, DPIV, sialyltransferase, DßM, carboxypeptidase H and cholinesterase.

Other preferred membrane proteins include gp55 or VSV glycoprotein, amyloid precursor protein (APP), ß-glycan, syndecan, DAF, VSG, CEA, prion protein, the Mcl-14 neutrophil adhesion protein or GP-2.

The drugs of the present invention (e.g. inhibitors of ADAM{met} and of the ADAM{met}-releasing interdomainase(s)) also find application in the treatment or prophylaxis of hypertension as angiotensin-converting enzyme (ACE) inhibitors.

The invention also contemplates an <u>in vitro</u> method of diagnosis (e.g. of multiple myeloma) comprising the step of detecting the expression of an ADAM gene (e.g. ADAM 12) in a selected class of cells (e.g. in myeloma cells).

The ADAM{met} of the invention may also be used in wound debridement (e.g. following burn injury).

The invention may also be used to regulate angiogenesis, neovascularization (e.g. of tumours), vascular proliferation in the eye and diabetes angiopathy.

The activity of ADAM{met} on various cell surface receptors may be exploited in the treatment or prophylaxis of various parasitic diseases which involve the interaction of parasite and receptor (e.g. malaria and diseases caused by trypanosomes, leishmania or amoebae). Here, the drug of the invention is used to prevent the interaction of the parasite with its cognate cellular receptor.

Exemplification

Brief description of the Figures

Figure 1 shows the sequence of the full length cDNA for *h*ADAM 12.

Figure 2 shows the predicted *h*ADAM 12 protein sequence for the full length *h*ADAM 12 cDNA. Highlighted areas identify the *h*ADAM 12{pro} domain (1), cysteine switch (2), the *h*ADAM 12{met} domain (3), the *h*ADAM 12{egf} domain (4) and *h*ADAM 12{tmt} domain (5). The *h*ADAM 12{dis} domain is underlined.

Figure 3 shows in schematic form the domain structure of *h*ADAM 12, including the metalloproteinase domain (*h*ADAM 12{met}), the disintegrin-like domain (*h*ADAM 12{dis}), a cysteine-rich region, an epidermal growth factor-like domain (*h*ADAM 12{egf}, stippled) and a putative transmembrane sequence (*h*ADAM 12{tmt}, TMT), with a transmembrane subdomain shown as a solid bar.

Figure 4 shows an alignment of protein sequences from *h*ADAM 12, atrolysin-E (ATR-E, reprolysin), HR1B (reprolysin), PH-30α, PH-30ß, EAP1, MDC and MS2.

Figure 5 shows an alignment of the disintegrin-like region. Conserved cysteine residues and ECD motif are highlighted. Bitistatin (Bit) is included in this line up to identify the disintegrin domain. The position of the RGD sequence in bitistatin is underlined.

Example 1: Cloning of *h*ADAM 12

Cell Culture

The human myeloma cell lines, U266-B1, HS-Sultan, ARH-77 and RPMI-8226 were obtained from the European Collection of Animal Cell Cultures (Porton Down, UK). All cell lines were maintained in RPMI 1640 (Life technologies)

supplemented with 10% foetal calf serum (Life technologies).

RNA Isolation

Total RNA was isolated from cells using a single step purification method based upon the method of Chomcynski and Sacchi (4). Briefly, cells (5 x 10^6) were pelleted by centrifugation, the supernatent removed and the cells lysed with TRIzol (Life technologies). Following addition of chloroform, RNA was separated into aqueous and organic phases by centrifugation at 12000 x g. RNA was recovered by precipitation with isopropanol, washed with 75% ethanol and resuspended in diethylpyrocarbonate treated water.

RT-PCR

Contaminating DNA was removed from RNA by incubating samples with DNase I for 1 hour at 37°C. RNA was reversed transcribed to cDNA using MMLV reverse transcriptase primed with random hexamers (Pharmacia). The PCR Primers used to amplify TIMP-3 were 5′ ATCACCTGGGTCATATGTGCAAGATCAAG 3′ and 5′ GATTCTCAGGGGTCTGTGGCATTGATG 3′. Products of the PCR reaction were electrophoresed on a 1.5% agarose gell in Tris-Borate-EDTA (TBE). Amplified products were excised and extracted from the gel using the Wizard DNA

purification system (Promega). Purified DNA was cloned into a pCRTMII vector (Invitrogen) following the manufacturers protocol.

DNA Sequencing

DNA sequencing was performed using a Sequenase Kit (USB Biochemicals) using synthetic oligonucleotide primers. Sequenced products were electrophoresed on an 8% denaturing acrylamide gel.

Northern Blot Analysis

15μm of RNA was electrophoresed on a 1% agarose-formaldehyde gel, transferred to a positively charged nylon membrane (Boehringer Mannheim) by capillary blotting and fixed by UV crosslinking (Stratalinker, Stratagene). Membranes were pre-hybridised for two hours in Easy-Hyb (Boehringer-Mannheim). cDNAs identified from the Expressed Sequence Tag (EST) database were excised, gel purified and α^{32}P-labelled using a random priming method (prime 1, Sigma). Hybridisation was performed overnight at 50°C. Membranes were washed at a final stingency of 0.1 x SSC, 0.1% SDS at 68°C. Membranes were exposed to autoradiography film at -70°C. Ethidium bromide staining of the 28S rRNA served to standardise RNA loading.

Library Screening

A human placental cDNA library (Stratagene) was plated according to manufacturers instructions and screened with cDNAs identified from the EST database. 10^7 individual clones were screened by filter hybridisation on Hybond N (Amersham). Overlapping positive clones were isolated, amplified and used as templates for DNA sequencing analysis.

Results

Two products were amplified by RT-PCR, one of approximately 200bp and the other of 220bp, in all cell lines examined. Translation, in all three reading frames, of the 200bp fragment revealed the presence of a putative cysteine rich open reading frame. The inferred protein sequence showed no similarity to any amino acid sequences deposited in the Swiss-Prot database. Screening of the EST database revealed two cDNA clones with 100% identity to the 200bp fragment, indicating that this fragment was likely to be expressed in human tissue. The two clones, H11999 and R31348 cloned from an infant brain library and a human placental library, respectively, were obtained from Research Genetics Incorporated (California, USA). H11999 was used to probe a Northern blot of RNA prepared from

human myeloma cell lines and shown to hybridise to RNA of approximately 3.8kbp.

Overlapping cDNAs were isolated from a human placental library by screening with HII999 and the DNA sequenced. A total of 3.5kbp of cDNA was isolated by this method. The predicted protein sequence for the full length cDNA is shown in Figure 2. The sequence encompasses an open reading frame of 2.2kbp with an inferred 3' untranslated region (UTR) of approximately 1.6kbp. This agrees with the Northern analysis indicating that the full length cDNA for this protein is likely to have been cloned. Thus, a novel human mRNA was identified whose potential translation products contain a number of characteristics in common with the class III reprolysins.

*h*ADAM 12 has significant amino acid similarity to a number of mammalian proteins with a variety of functions. These include ADAM 8 (MS2), a cell surface antigen of approximately 89kD, expressed in the murine macrophage lineage. This shows significant identity with *h*ADAM 12; however, the authors omitted to identify the characteristic zinc binding motif characteristic of the metalloproteinases. *h*ADAM 12 also has significant identity with both subunits of ADAM 2 (PH-30), which is an heterodimeric protein implicated in sperm-egg fusion. The

α subunit contains the consensus zinc-binding motif which aligns with *h*ADAM 12, whereas, the ß subunit, although not containing this region, contains a disintegrin-like sequence which also shares considerable identity with *h*ADAM 12.

Another similar protein has been identified in the epididymis of a number of mammalian species. This protein, ADAM 7 (epididymal apical protein 1 or EAP1), has also been proposed to contain a metalloproteinase domain; however, on the basis of the presented sequence it is unlikely to be catalytically active since it lacks the glutamic acid residue within the zinc-binding motif. ADAM 7 also contains a disintegrin-like domain with similarity to *h*ADAM 12.

ADAM 11 (or MDC), a recently described tumour suppressor gene, shown to be somatically rearranged in primary breast cancer, also shows significant similarities to *h*ADAM 12. This protein was also suggested to contain a metalloproteinase domain; however, the presented sequence does not appear to contain a characteristic zinc-binding motif, although it does contain a putative cysteine switch and may represent a member of a novel class of zinc-binding metalloproteinases.

Example 2: Cloning of *b*ADAM 12

Isolation of chondrocytes

Chondrocytes were isolated following collagenase digestion of cartilage explants cut from fresh tissue. Culture conditions were as published in Buttle et al. (1993) Arthritis Rheum 36, pages 1709-1717.

RNA Isolation

Total RNA was isolated from cells using a single step purification method based upon the method of Chomcynski and Sacchi (1987), Anal. Biochem. Vol. 162: 156-159. Briefly, cells (5×10^6) were pelleted by centrifugation, the supernatent removed and the cells lysed with TRIzol (Life technologies). Following addition of chloroform, RNA was separated into aqueous and organic phases by centrifugation at 12000 x g. RNA was recovered by precipitation with isopropanol, washed with 75% ethanol and resuspended in diethylpyrocarbonate treated water.

RT-PCR

Contaminating DNA was removed from RNA by incubating samples with DNase I for 1 hour at 37°C. RNA was reversed

transcribed to cDNA using MMLV reverse transcriptase primed with random hexamers (Pharmacia). The PCR Primers used were based upon the human ADAM sequence.

Products of the PCR reaction were electrophoresed on a 1.5% agarose gell in Tris-Borate-EDTA (TBE). Amplified products were excised and extracted from the gel using the Wizard DNA purification system (Promega). Purified DNA was cloned into a pCRTMII vector (Invitrogen) following the manufacturer's protocol.

DNA Sequencing

DNA sequencing was performed using a Sequenase Kit (USB Biochemicals) using synthetic oligonucleotide primers. Sequenced products were electrophoresed on an 8% denaturing acrylamide gel.

DNA sequence of bovine ADAM12

The partial sequence was determined to comprise the nucleotide sequence:

GCCAGTGTGATGGATATCTGCAGAATTCGGCTTGAATTCTCAGGGGTCTTTGGCATT
GATGGGAACTGCTGAGGTTGCTTGGGTGCATAGGTTGGGACTGCAAATCTGCTGTGG
ATATATAGGAACTTCTCTGGGAGGGGTCACTGGAGAAACATATCGAGGACACACCCC

CCTGTCTAGAAGGGTTTGGTT

CLAIMS:

1. Isolated ADAM 12 protein.

2. Isolated protein comprising or consisting essentially of: (a) ADAM 12{pro}; and/or

(b) ADAM 12{met}; and/or

(c) ADAM 12{dis}; and/or

(d) ADAM 12{fus}; and/or

(e) ADAM 12{egf}; and/or

(f) ADAM 12{tmt}.

3. Isolated protein according to claim 2 comprising or consisting essentially of:

(a) ADAM 12{pro}; or

(b) ADAM 12{met}; or

(c) ADAM 12{pro} and ADAM 12{met}; or

(d) ADAM 12{pro}, ADAM 12{met} and ADAM 12{dis}; or

(e) ADAM 12{met} and ADAM 12{dis}; or

(f) ADAM 12{dis}; or

(g) ADAM 12{egf}; or

(h) ADAM 12{met} and ADAM 12{egf}; or

(i) ADAM 12{met}, ADAM 12{dis} and ADAM 12{egf}; or

(j) ADAM 12{pro}, ADAM 12{met}, ADAM 12{dis} and ADAM 12{egf};or

(k) ADAM 12{dis} and ADAM 12{fus}; or

(l) ADAM 12{met}, ADAM 12{dis} and ADAM 12{fus}.

4. A species variant (for example *h*ADAM 12 or *b*ADAM 12), homologue, derivative, mutein or equivalent of the protein as defined in any one of claims 1 to 3.

5. A pharmaceutical composition comprising the protein, species variant, homologue, derivative, mutein or equivalent thereof as defined in any one of claims 1 to 4.

6. A pharmaceutical composition comprising an ADAM protein or an isolated ADAM protein which is:

(a) for use in therapy, diagnosis or prophylaxis; or

(b) in a pharmaceutical excipient, a unit dosage form or in a form suitable for local or systemic administration.

7. Isolated protein consisting essentially of:

(a) ADAM{pro}; and/or

(b) ADAM{met}; and/or

(c) ADAM{dis}; and/or

(d) ADAM{fus}; and/or

(e) ADAM{egf}; and/or

(f) ADAM{tmt},

for example forming part of a pharmaceutical composition or for use in therapy, diagnosis or prophylaxis.

8. Isolated protein according to claim 7 consisting essentially of:

(a) ADAM{pro}; or

(b) ADAM{met}; or

(c) ADAM{pro} and ADAM{met}; or

(d) ADAM{pro}, ADAM{met} and ADAM{dis}; or

(e) ADAM{met} and ADAM{dis}; or

(f) ADAM{dis}; or

(g) ADAM{egf}; or

(h) ADAM{met} and ADAM{egf}; or

(i) ADAM{met}, ADAM{dis} and ADAM{egf}; or

(j) ADAM{pro}, ADAM{met}, ADAM{dis} and ADAM{egf},

(k) ADAM{dis} and ADAM{fus},

(l) ADAM{met}, ADAM{dis} and ADAM{fus}.

for example forming part of a pharmaceutical composition or for use in therapy, diagnosis or prophylaxis.

9. The isolated protein of any one of claims 1-8 wherein the ADAM{fus} is associated with the cysteine repeat pattern Cys-5/6X-Cys-4X-Cys-14X-Cys-12/13X-Cys-9/10/11X-Cys-6X-Cys-4X-Cys, or a functionally equivalent repeat pattern.

10. A pharmaceutical composition comprising or consisting essentially of an inhibitor of an ADAM{met}.

11. The composition of claim 10 which:

(a) comprises or consists essentially of an ADAM{pro} (for example the ADAM{pro} cysteine switch); or

(b) comprises or consists essentially of an ADAM{egf}; or

(c) is an antibody to an ADAM{met}; or

(d) is a pseudosubstrate of an ADAM{met}; or

(e) binds to and blocks an ADAM{met} effector site; or

(f) is a reprolysin inhibitor or derivative thereof; or

(g) comprises or consists essentially of the amino acid sequence motif PXXCGV, or a functional equivalent thereof; or

(h) comprises or consists essentially of the amino acid sequence motif PLKCGV, or a functional equivalent thereof; or

(i) is a peptide-hydroxamate; or

(j) is a pyroglutamate peptide; or

(k) is oprin, a member of the oprin family or a homologue, derivative, mutein or equivalent thereof; or

(l) is an alpha 1B glycoprotein or a homologue, derivative, mutein or equivalent thereof; or

(m) is a macroglobulin (e.g. alpha 2 macroglobulin) or a homolgue, derivative, mutein or equivalent thereof; or

(n) ligands the active site Zn ion of the ADAM{met}; or

(o) is all or a portion of the high-cysteine region.

12. A pharmaceutical composition comprising or consisting essentially of an inhibitor (for example an agonist or antagonist) of an ADAM{dis}.

13. The composition of claim 12 which:

(a) is an ADAM{dis} receptor mimetic (e.g. an integrin mimetic);

(b) is an ADAM{dis} receptor binding sequence mimetic (e.g. a peptide comprising or consisting essentially of the amino acid sequence ECD); or

(c) is an antibody to an ADAM{dis}.

14. The composition of claim 13(b) which comprises or consists essentially of an ADAM{dis} receptor binding sequence mimetic wherein the ECD sequence is within a rigid disulfide loop, a cyclic peptide or associated with an ADAM{fus}.

15. The composition of any one of claims 10-14 wherein the ADAM is ADAM 12.

16. The composition of any one of claims 10-15 wherein the

ADAM{fus} domain is associated with the cysteine repeat pattern Cys-5/6X-Cys-4X-Cys-14X-Cys-12/13X-Cys-9/10/11X-Cys-6X-Cys-4X-Cys, or a functionally equivalent repeat pattern.

17. The composition of any one of claims 10-16 which is:

(a) for use in therapy, diagnosis or prophylaxis; or

(b) in a pharmaceutical excipient, a unit dosage form or in a form suitable for local or systemic administration.

18. Isolated ADAM interdomainase.

19. The interdomainase of claim 18 which cleaves at the:

(a) ADAM{pro}-ADAM{met} interdomain boundary; or

(b) ADAM{met}-ADAM{dis} interdomain boundary; or

(c) ADAM{dis}-ADAM{fus} interdomain boundary; or

(d) ADAM{fus}-ADAM{egf} interdomain boundary; or

(e) ADAM{egf}-ADAM{tmt} interdomain boundary.

20. The interdomainase of claim 18 or 19 wherein the ADAM is ADAM 12.

21. An inhibitor of the interdomainase of any one of claims 18-20.

22. The inhibitor of claim 21 which is peptide (e.g. a peptide comprising a tetra and/or dibasic residue) consisting essentially of an interdomain amino acid sequence selected from:

(a) the ADAM{pro}-ADAM{met} interdomain amino acid sequence; or

(b) the ADAM{met}-ADAM{dis} interdomain amino acid sequence; or

(c) the ADAM{dis}-ADAM{fus} interdomain amino acid sequence; or

(d) the ADAM{fus}-ADAM{egf} interdomain amino acid sequence; or

(e) the ADAM{egf}-ADAM{tmt} interdomain amino acid sequence,

or functionally equivalent amino acid sequences derived therefrom or modelled thereon.

23. The interdomainase or inhibitor of any one of claims 18-22 which is:

(a) for use in therapy, diagnosis or prophylaxis; or

(b) in a pharmaceutical excipient, a unit dosage form or in a form suitable for local or systemic administration.

24. A pharmaceutical composition comprising the interdomainase or inhibitor of any one of claims 18-22.

25. Isolated nucleic acid (for example DNA, e.g. cDNA) encoding the protein of any one of claims 1-4 and 7-9 or the interdomainase of any one of claims 18-20, or a nucleic acid probe which is selectively hybridizable therewith.

26. A vector comprising the nucleic acid of claim 25.

27. A host cell comprising the vector of claim 26.

28. A process for producing the protein of any one of claims 1-4 and 7-9 or the interdomainase of any one of claims 18-20 comprising the steps of:

(a) culturing the host cell of claim 27, and

(b) purifying the protein or interdomainase from the cultured host cells (e.g. from a culture supernatant or cell fraction).

29. A process for producing the protein of any one of claims 1-4 and 7-9 comprising the steps of:

(a) probing a gene library with a nucleic acid probe which is selectively hybridizable with the nucleic acid of claim 25 (for example having a sequence which is comprised in a gene corresponding to the sequence shown in Fig. 1), to produce a signal which identifies a gene that selectively hybridizes to the probe;

(b) expressing the gene identified in step (a) (for

example by cloning into a host cell) to produce the protein.

30. A protein or interdomainase obtainable by the process of claim 28 or 29.

31. A vector for altering the expression of an ADAM (e.g. ADAM 12) in vivo comprising DNA as defined in claim 25 and/or upstream or downstream DNA.

32. A pharmaceutical composition comprising the vector of claim 31.

33. A method for screening for therapeutically active drugs comprising the steps of:

(a) contacting a candidate drug with the protein of any one of claims 1-4 and 7-9 or the interdomainase of any one of claims 18-20, and

(b) determining whether binding between the candidate drug and protein/interdomainase occurs, wherein binding is indicative of a therapeutically active drug.

34. A method for evaluating the therapeutic activity of a drug comprising the steps of:

(a) contacting a candidate drug with the protein of

any one of claims 1-4 and 7-9 or the interdomainase of any one of claims 18-20, and

(b) measuring the binding affinity of the candidate drug for the protein or interdomainase,

the drug for example being an inhibitor of an ADAM{met} and the protein comprising an ADAM{met} (e.g. ADAM 12{met}).

35. A method for synthesizing a therapeutically active drug comprising the steps of:

(a) generating a three-dimensional model of the active site (e.g. the catalytic or ligand binding site) of the protein of any one of claims 1-4 and 7-9 or the interdomainase of any one of claims 18-20 (e.g. by computer analysis of its amino-acid sequence or by X ray crystallography of the protein/interdomainase or fragment thereof), and

(b) modelling the drug with reference to the three-dimensional model generated in step (a),

the drug for example being an inhibitor of an ADAM{met} and the protein comprising an ADAM{met} (e.g. ADAM 12{met}).

36. A therapeutically active drug which has been screened, evaluated or synthesised by the methods of claims 33-35.

37. An antibody (e.g. a monoclonal antibody) which binds (for example, specifically) to the protein of any one of

claims 1-4 and 7-9 or the interdomainase of any one of claims 18-20, or a derivative thereof.

38. A test kit comprising the protein of any one of claims 1-4 and 7-9 or the interdomainase of any one of claims 18-20, for example for use in the method of claim 33 or 34.

39. A test kit according to claim 38 wherein: (i) the protein is bound to a solid support and/or (ii) the kit further comprises a labelled (e.g. radiolabelled or fluorescently labelled) protein substrate, receptor or ligand and/or (iii) the kit further comprises the antibody of claim 37.

40. The invention as defined in any one of the preceding claims for use in therapy, prophylaxis or diagnosis.

41. The invention of claim 40 wherein the therapy, prophylaxis or diagnosis is of:

(a) disorders or diseases of connective tissue; or

(b) disorders or diseases of the skeletal system (e.g. bone); or

(c) inflammatory diseases or disorders; or

(d) cancer (e.g. multiple myeloma); or

(e) coagulopathies; or

(f) haemorrhagic disorders or diseases; or

(g) amyloidosis (e.g. Alzheimer's disease).

42. The invention of claim 41(a) wherein the disorder or disease is:

(a) that requiring regulation of aggrecan degradation (e.g. the regulation of aggrecanase activity);

(b) that requiring the regulation of cartilage breakdown;

(c) that requiring the prevention or reduction of cartilage breakdown;

(d) arthritides, for example osteoarthritis or rheumatoid arthritis.

43. The invention of claim 41(b) wherein the disorder or disease is:

(a) that characterized by bone resorption; or

(b) osteoporosis, for example postmenopausal osteoporosis; or

(c) Paget's disease; or

(d) metastatic bone disease; or

(e) myeloma associated bone disease.

44. The invention of claim 41(c) wherein the disorder or disease is:

(a) mediated by proinlammatory cytokines; and/or

(b) mediated by cell (e.g. macrophage) infiltration;

and/or

(c) rheumatoid arthritis.

45. The invention of claim 41(d) wherein the cancer therapy, prophylaxis or diagnosis is:

(a) the detection or prevention of tumour progression;

(b) the detection or prevention of tumour metastasis.

46. The invention of claim 41(e) wherein the coagulopathy is a thrombo-embolic disorder.

47. The invention of any one of claims 1 to 39 for:

(a) regulating, promoting or reducing membrane protein shedding; or

(b) regulating, promoting or reducing membrane protein processing; or

(c) regulating, promoting or reducing ADAM processing; or

(d) regulating, promoting or reducing cell-cell adhesion/contact/communication (e.g. juxtacrine stimulation); or

(e) regulating, promoting or reducing aggrecan degradation, e.g. via the regulation, promotion or reduction of aggrecanase activity; or

(f) regulating, promoting or reducing the degradation of basmement membrane components; or

(g) regulating, promoting or reducing osteoclast precursor fusion; or

(h) regulating, promoting or reducing collagen type II degradation and/or removal; or

(i) regulating, promoting or reducing the activation of a matrix metalloprotease (MMP).

48. The invention of claim 47(a) or 47(b) wherein the membrane protein is:

(a) a receptor; or

(b) a receptor ligand; or

(c) a cell adhesion molecule; or

(d) a leukocyte antigen; or

(e) an ectoenzyme.

49. The invention of claim 48(a) wherein the receptor is a cytokine receptor, for example a receptor for any one of TNF, IL-6, CSF-1, NGF, EGF, GH.

50. The invention of claim 48(a) wherein the receptor is a receptor for any one of transferrin, poly-Ig and folate.

51. The invention of claim 48(b) wherein the receptor ligand is a cytokine (e.g. a lymphokine or monokine) or growth factor, for example any one of Steel factor, TNF (e.g. TNFα), TGF-α, CSF-1, Fas ligand (FasL) or *c-kit* ligand.

52. The invention of claim 48(c) wherein the cell adhesion molecule is any one of gp 100, Leu 8, ELAM-1, GMP-140,, glycoprotein Ib and neural cell adhesion molecule (NCAM).

53. The invention of claim 48(d) wherein the leukocyte antigen is any one of immunoglobulin, CD8, class I MHC, IL 2 receptor, IL 4 receptor, CD16, CD23 and CD14.

54. The invention of claim 48(e) wherein the ectoenzyme is any one of angiotensin-converting enzyme (ACE), NEP 24.11, DPIV, sialyltransferase, DßM, carboxypeptidase H and cholinesterase.

55. The invention of claim 47(a) or 47(b) wherein the membrane protein is gp55 or VSV glycoprotein, amyloid precursor protein (APP), ß-glycan, syndecan, DAF, VSG, CEA, prion protein, the Mcl-14 neutrophil adhesion protein or GP-2.

56. An <u>in vitro</u> method of diagnosis (e.g. of multiple myeloma) comprising the step of detecting the expression of an ADAM gene (e.g. ADAM 12) in a selected class of cells (e.g. in myeloma cells).

TCAGGGCTAT GGTGGAGGGAG TTCATAATTC ATCCATTGCT CTTAGCGACT
GTTTTGGACT CAGAGGATTG CTGCATTTAG AGAATGCGAG TTATGGGATT
GAACCCCTGC AGAACAGCTC TCATTTTGAG CACATCATTT ATCGAATGGA
TGATGTCTAC AAAGAGCCTC TGAAATGTGG AGTTTCCAAC AAGGATATAG
AGAAAGAAAC TGCAAAGGAT GAAGAGGAAG AGCCTCCCAG CATGACTCAG
CTACTTCGAA GAAGAAGAGC TGTCTTGCCA CAGACCCGGT ATGTGGAGCT
GTTCATTGTC GTAGACAAGG AAAGGTATGA CATGATGGGA AGAAATCAGA
CTGCTGTGAG AGAAGAGATG ATTCTCCTGG CAAACTACTT GGATAGTATG
TATATTATGT TAAATATTCG AATTGTGCTA GTTGGACTGG AGATTTGGAC
CAATGGAAAC CTGATCAACA TAGTTGGGGG TGCTGGTGAT GTGCTGGGGA
ACTTCGTGCA GTGGCGGGAA AAGTTTCTTA TCACACGTCG GAGACATGAC
AGTGCACAGC TAGTTCTAAA GAAAGGTTTT GGTGGAACTG CAGGAATGGC
ATTTGTGGGA ACAGTGTGTT CAAGGAGCCA CGCAGGCGGG ATTAATGTGT
TTGGACAAAT CACTGTGGAG ACATTTGCTT CCATTGTTGC TCATGAATTG
GGTCATAATC TTGGAATGAA TCACGATGAT GGGAGAGATT GTTCCTGTGG
AGCAAAGAGC TGCATCATGA ATTCAGGAGC ATCGGGTTCC AGAAACTTTA
GCAGTTGCAG TGCAGAGGAC TTTGAGAAGT TAACTTTAAA TAAAGGAGGA
AACTGCCTTC TTAATATTCC AAAGCCTGAT GAAGCCTATA GTGCTCCCTC
CTGTGGTAAT AAGTTGGTGG ACGCTGGGGA AGAGTGTGAC TGTGGTACTC
CAAAGGAATG TGAATTGGAC CCTTGCTGCG AAGGAAGTAC CTGTAAGCTT
AAATCATTTG CTGAGTGTGC ATATGGTGAC TGTTGTAAAG ACTGTCGGTT
CCTTCCAGGA GGTACTTTAT GCCGAGGAAA AACCAGTGAG TGTGATGTTC
CAGAGTACTG CAATGGTTCT TCTCAGTTCT GTCAGCCAGA TGTTTTTATT
CAGAATGGAT ATCCTTGCCA GAATAACAAA GCCTATTGCT ACAACGGCAT
GTGCCAGTAT TATGATGCTC AATGTCAAGT CATCTTTGGC TCAAAAGCCA
AGGCTGCCCC CAAAGATTGT TTCATTGAAG TGAATTCTAA AGGTGACAGA
TTTGGCAATT GTGGTTTCTC TGGCAATGAA TACAAGAAGT GTGCCACTGG
GCTGTCACTG AAATTTCATG CTCCATTCTG AGCACTATGC TTCAAGAGGC
AGTGAGGCAA ACTGGAACAT ATTTAGGAGG GAGCGTCCTT GTTGCATGAA
AAGTGATTGT AGGATAGTGA CCTGGTGAAG AATGCTTTGT GTGGAAAGCT
TCAGTGTGAG AATGTACAAG AGATACCTGT ATTTGGAATT GTGCCTGCTA
TTATTCAAAC GCCTAGTCGA GGCACCAAAT GTTGGGGTGT GGATTTCCAG
CTAGGATCAG ATGTTCCAGA TCCTGGGATG GTTAACGAAG GCACAAAATG
TGGTGCTGGA AAGATCTGTA GAAACTTCCA GTGTGTAGAT GCTTCTGTTC
GAATTATGA CTGTGATGTT CAGAAAAAGT GTCATGGACA TGGGGTATGT
ATAGCAATA AGAATTGTCA CTGTGAAAAT GGCTGGGCTC CCCCAAATTG
TGAGACTAAA GGATACGGAG GAAGTGTGGA CAGTGGACCT ACATACAATG
AAATGAATAC TGCATTGAGG GACGGACTTC TGGTCTTCTT CTTCCTAATT
GTTCCCCTTA TTGTCTGTGC TATTTTTATC TTCATCAAGA GGGATCAACT
GTGGAGAAGC TACTTCAGAA AGAAGAGATC ACAAACATAT GAGTCAGATG
GCAAAAATCA AGCAAACCCT TCTAGACAGC CGGGGAGTGT TCCTCGACAT
GTTTCTCCAG TGACACCTCC CAGAGAAGTT CCTATATATG CAAACAGATT
TGCAGTACCA ACCTATGCAG CCAAGCAACC TCAGCAGTTC CCATCAAGGC
CACCTCCACC ACAACCGAAA GTATCATCTC AGGGAAACTT AATTCCTGCC
CGTCCTGCTC CTGCACCTCC TTTATATAGT TCCCTCACTT GATTTTTTTA
ACCTTCTTTT TGCAAATGTC TTCAGGGAAC TGAGCTAATA CTTTTTTTTT
TTCTTGATGT TTTCTTGAAA AGCCTTTCTG TTGCAACTAT GAATGAAAAC
AAAACACCAC AAAACAGACT TCACTAACAC AGAAAAACAG AAACTGAGTG
TGAGAGTTGT GAAATACAAG GAAATGCAGT AAAGCCAGGG AATTTACAAT
AACATTTCCG TTTCCATCAT TGAATAAGTC TTATTCAGTC ATCGGTGAGG
TTAATGCACT AATCATGGAT TTTTTGA

FIG. 1

1 2

MWREFIIHPL **LLATVLDLRG** **LLHL**ENASYG IEPLQMSSHF EHIIYRMDDV YKE**PLKCGVS**

NKDIEKETAK DEEEEPPSMT QLLRRRRAVL PQTRYVELFI VVDKERYDMM GRNQTAVREE

MILLANYLDS MYIMLNIRIV LVGLEIWTNG NLINIVGGAG DVLGNFVQWR EKFLITRRRH

3

DSAQLVLKKG FGGTAGMAFV GTVCSRSHAG GINVFGQITV ETFASIVA**HE** **LGH**NLGMNHD

DGRDCSCGAK SCIMNSGASG SRNFSSCSAE DFEKLTLNKG GNCLLNIPKP DEAYSAPSCG

NKLVDAGEEC DCGTPKECEL DPCCEGSTCK LKSFAECAYG DCCKDCRFLP GGTLCRGKTS

ECDVPEYCNG SSQFCQPDVF IQNGYPCQNN KAYCYNGMCQ YYDAQCQVIF GSKAKAAPKD

CFIEVNSKGD RFGNCGFSGN EYKKCATGLS LKFHAPFLST MLQEAVRQTG TYLGGSVCCM

KSDCRIVTLV KNALCGKLQC ENVQEIPVFG IVPAIIQTPS RGTKCWGVDF QLGSDVPDPG

4

MVNEGTKCGA GKICRNFQCV DASVLNYDCD VQK**KCHGHGV** **CNSNKNCHCE** NGWAPPN**C**ET

5

KGYGGSVDSG PTYNEMNTAL RDGLLVFFFL IVPLIVCDYF YLHQEGSTVE KLLQKEEITN

FIG. 2

FIG. 3

A METALLOPROTEINASE DOMAIN

MCMP	IVA**HELGHNLGMNHDDGRDCSCGAK**.....SCIM
ATR-E	TMT**HELGHNLGIHHD**.TDSCS.GGY.....SCIM
HR1B	IMT**HEMGHNLGIPHD**.GNSCTCGGF.....PCIM
PH-30a	LLV**HELGHNLGIRHDHSA**.CVCRDK.....HSCLM
PH-30b	ILVQLLSVSMGIAYDNADLCRCRGA.....ICLM
EAP1	RMA**HQLGHNLGMQHD**.EFPCTCPS.....GKCVM
MDC	TLAQTLGQNLGMMWMKHRSSAGDCKCPDIWLGCIM
MS2	TMA**HELGHNLGMSHDEDI**PGCYCPE.PREGGGCIM

FIG. 4

FIG. 5

B DISINTEGRIN-LIKE DOMAIN

MCMP	NKLVDAGEEC	DCGTPKECEL	DPCCEGSTCK	LKSFAECAYG
ATR-E	NELLEAGIEC	DCGSLEN...	.PCCYATTCK	MRPGSQCAEG
HR1B	NELLEAGEEC	DCGSPENCQ.	YQCCDAASCK	LHSWVKCESG
PH-30a	NGVVEESEQC	DCGV..NCDT	SECCDQ.ACN	LKGNATCSNE
PH-30b	NNRVEQGEDC	DCGSQEECQ.	DTCCDAATCR	LKSTSRCAQG
EAP1	NKKLDEGEEC	DCGPPQECT.	NPCCDAHTCV	LKPGFTCAEG
MDC	NGFVEAGEEC	DCGSVQECSR	AGGNCCKKCT	LTHDAMCSDG
MS2	NLFVEHGEQC	DCGTPQDCQN	P.CCNATTCQ	LVKGAECASG
Bit	NKILEQGEDC	DCGSPANCQ.	DQCCNATTCK	LTPGSQCNHG

MCMP	DCCKDCRFLP	GGTLCRGKTS	ECDVPEYCNG	SSQFCQPDVF
ATR-E	LCCDQCRFMK	KGTVCRVSMV	DRN.DDTCTG	QSADCPRNGL
HR1B	ECCDQCRFRT	AGTECRAAES	ECDIPESCTG	QSADCPTDRF
PH-30a	LCCSDCQYKN	SGYLCRPSVG	PCDLPEYCTG	QSGKCPLDTY
PH-30b	PCCNQCEFKT	KGEVCRESTD	ECDLPEYCNG	SSGACQEDLY
EAP1	ECCESCQIKK	AGSICRPAED	ECDFPEMCTG	HSPACPKDQF
MDC	LCCRRCKYEP	RGVSCREAVN	ECDIAETCTG	DSSQCPPNLH
MS2	TCCHECKVKP	AGEVCRLSKD	KCDLEEFCDG	RKPTCPEDAF
Bit	.CCDQCKFKK	ARTVCRIARG	DWN.DDYCTG	KSSDCPWNH

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/705, C12N 15/58, A61K 38/17, 38/48, G01N 33/68, C07K 16/40, 16/28, C12Q 1/37, 1/68, C12N 15/62, A61K 39/395, C12N 9/64

A3

(11) International Publication Number: **WO 97/40072**

(43) International Publication Date: 30 October 1997 (30.10.97)

(21) International Application Number: PCT/GB97/01067

(22) International Filing Date: 16 April 1997 (16.04.97)

(30) Priority Data:
9608130.2 19 April 1996 (19.04.96) GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
26 March 1998 (26.03.98)

(54) Title: ADAM PROTEINS AND USES THEREOF

(57) Abstract

Described are ADAM 12 proteins, species variants, homologues, allelic forms, mutant forms, derivatives, muteins and equivalents thereof, and various individual domains of ADAM 12 proteins, various domain combinations, inhibitors thereof and various forms of therapy, diagnosis and prophylaxis based thereon. Various therapeutic, diagnostic and prophylatic applications of proteins of the ADAM (reprolysin) family in general, as well as their individual domains, domain combinations, inhibitors and other materials based thereon, are also described.

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INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/GB 97/01067

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C12N15/58 A61K38/17 A61K38/48
G01N33/68 C07K16/40 C07K16/28 C12Q1/37 C12Q1/68
C12N15/62 A61K39/395 C12N9/64

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WESKAMP G ET AL: "MDC9, A WIDELY EXPRESSED CELLULAR DISINTEGRIN CONTAINING CYTOPLASMIC SH3 LIGAND DOMAINS" THE JOURNAL OF CELL BIOLOGY, vol. 132, no. 4, February 1996, pages 717-726, XP000644308 see the whole document ---	1-4, 25-30,37
A	S. YOSHIDA ET AL: "Molecular cloning of cDNA encoding MS2 antigen, a novel surface antigen strongly expressed in murine monocytic lineage" INTERNATIONAL IMMUNOLOGY, vol. 2, no. 6, June 1990, pages 585-591, XP002044200 see the whole document ---	1-17, 25-56

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
21 October 1997	18.02.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	LE CORNEC N.D.R.

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INTERNATIONAL SEARCH REPORT

Intern nal Application No
PCT/GB 97/01067

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	T.G. WOLSBERG ET AL: "ADAM, a novel family of membrane proteins containing A Disintegrin And Metalloprotease domain : Multipotential functions in cell-cell and matrix interactions" JOURNAL OF CELL BIOLOGY, vol. 131, no. 2, October 1995, THE ROCKEFELLER UNIVERSITY PRESS, pages 275-278, XP002044201 cited in the application see the whole document	1-17, 25-56
A	WO 95 35118 A (MERCK & CO., INC.) 28 December 1995 see claims; examples 1-5 see page 10, line 27 - page 16, line 25	
P,X	N. MCKIE ET AL: "Cloning of a novel membrane-linked metalloproteinase from human myeloma cells" BIOCHEMICAL JOURNAL, vol. 318, no. 2, 1 September 1996, pages 459-462, XP002044202 see the whole document	1-4, 25-30
P,X	ARI-PEKKA J. HUOVILA ET AL: "ADAMs and cell fusion" CURRENT OPINION IN CELL BIOLOGY, vol. 8, no. 5, October 1996, pages 692-699, XP002044203 see the whole document	1-4
P,X	N. MCKIE ET AL: "Expression of Members of a novel Membrane linked Metalloproteinase family (ADAM) in human articular chondrocytes" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS., vol. 230, no. 2, 13 January 1997, ORLANDO, FL US, pages 335-339, XP002044204 see the whole document especially figure 3 page 337	1-4, 25-30
T	E. WU ET AL: "expression of members of the novel membrane linked Metalloproteinase family ADAM in cells derived from a range of haematological malignancies" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS., vol. 235, no. 2, 18 June 1997, ORLANDO, FL US, pages 437-442, XP002044205	

INTERNATIONAL SEARCH REPORT

In abonal application No.
PCT/GB 97/01067

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 33-34 (as far as in vivo methods are concerned) is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

For further information see continuation sheet(s) PCT/ISA/210

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Repon covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-5,15 complete and 6-14,16-17,25-56 partial

Remark on Protest

[ ] The additional search fees were accompanied by the applicant s protest.

[ ] No protest accompanied the payment of additional search fees

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: (1-5,15) completely; (6-14,16-17,25-56) partially

ADAM 12,fragments, derivatives, inhibitors, antibody immunoreactive with ADAM 12, encoding DNA, process of production of said protein by genetic engineering, pharmaceutical composition, use in a method of treatment and in a method of screening.

2. Claims: (6-8,40-55) partially

ADAM protein other than ADAM 12 or a fragment thereof in a pharmaceutical composition .

3. Claims: (9,40-55)partially

ADAM protein other than ADAM 12 or a fragment thereof wherein ADAM(fus) is associated with a cystein repeat and its use in therapy, prophylaxis or diagnosis.

4. Claims: (10-14,16-17,40-55) partially

Pharmaceutical composition containing an Inhibitor of ADAM(met) or an inhibitor of ADAM(dis) as far as it is not covered by the previous inventions and its use in therapy, prophylaxis or diagnosis.

5. Claims: (18-24) completely; (25-28,30,33-55) partially

ADAM interdomainase, encoding DNA, process to produce said protein by genetic engineering, an inhibitor of the enzyme, an antibody immunoreactive with the interdomainase, use of the enzyme in a method of screening, in a pharmaceutical composition, in therapy, diagnosis or prophylaxis.

6. Claims: (25-32) partially

DNA encoding ADAM protein other than ADAM 12 , a vector containing it, a host cell transformed, use in a process of production of the protein by genetic enginering and the protein obtained.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. Claims: (33-36,38-55) partially

Method of screening, of evaluation and of synthesis of a therapeutically active drug using an ADAM protein other than ADAM 12 and the drug obtained as far as it is not covered by previous inventions.

8. Claims: (37,39,40-55) partially

Antibody immunoreactive with ADAM protein other than ADAM 12 and its use in a test kit, in therapy, prophylaxis or diagnosis as far as it is not covered by previous inventions.

9. Claim : 56 partially

In vitro method of diagnosis by detecting the expression of an ADAM gene other than ADAM 12 in myeloma cells.

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern: ...al Application No
PCT/GB 97/01067

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9535118 A	28-12-95	US 5693496 A	02-12-97
		AU 2769595 A	15-01-96
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		EP 0771212 A	07-05-97